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## ASSESSING AND MITIGATING LAWN INSECTICIDE HAZARDS TO BEES AND OTHER BENEFICIAL INVERTEBRATES

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Dr. Chuck Fox, Director of Graduate Studies

ASSESSING AND MITIGATING LAWN INSECTICIDE HAZARDS TO BEES AND  
OTHER BENEFICIAL INVERTEBRATES

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DISSERTATION

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A dissertation submitted in partial fulfillment of the requirements for the degree of  
Doctor of Philosophy in the College of Agriculture, Food, and the Environment at the  
University of Kentucky

By

Jonathan Lane Larson

Lexington, Kentucky

Director: Dr. Daniel A. Potter

Lexington, Kentucky

2014

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## ABSTRACT OF DISSERTATION

### ASSESSING AND MITIGATING LAWN INSECTICIDE HAZARDS TO BEES AND OTHER BENEFICIAL INVERTEBRATES

Turfgrass settings, including lawns, golf courses, and sports fields, support many beneficial invertebrates that provide important ecosystem services. These non-target organisms and their associated predation, decomposition, and pollination services can be disrupted by the use of certain insecticides. I compared the ecotoxicity of representatives from three major turf insecticide groups, the neonicotinoids, premix formulations, and the anthranilic diamides, in lab and field realistic settings in order to inform industry initiatives towards environmental sustainability.

In lab and field bioassays clothianidin, a neonicotinoid, and a premix clothianidin/pyrethroid spray were acutely toxic to beneficial insects. Populations of predators, springtails, and earthworms, as well as parasitism, predation, and decomposition rates were all reduced. In contrast, chlorantraniliprole, a novel anthranilic diamide with a similar spectrum of pests controlled, had no apparent impact on natural enemies, decomposers, or ecosystem services. This newer class is a good fit for industry initiatives to use relatively less toxic pesticides, with the caveat that golf course superintendents may see secondary pest outbreaks of ants and earthworms.

Bumble bee colonies exposed to clothianidin-treated white clover for two weeks suffered acute effects including increased mortality of workers and decreases in the number of honeypots constructed in the hive. When hives were exposed to clothianidin treated clover for six days and then allowed to develop naturally over six weeks they exhibited delayed weight gain and produced no new queens. Colonies exposed to chlorantraniliprole-treated flowers suffered no observable adverse effects. When treated blooms were mowed, colonies exposed to newly-formed blooms exhibited no ill effects. After a single mowing neonicotinoid residues in clover nectar were reduced from > 2000 ng/g, to < 10 ng/g. Residues of imidacloprid were also short-lived in guttation water.

Some 50 species of bees and other pollinators were collected from flowering white clover and dandelions in lawns across an urbanization gradient. Such weeds, an underappreciated resource for urban bees, could play a role in pollinator conservation if tolerated and not over-sprayed with broad-spectrum insecticides. Informing the public about the potential benefits these weeds could have for pollinators may help lead to more environmentally conscious management decisions.

Key words: chlorantraniliprole, pollinators, ecotoxicology, neonicotinoids, turfgrass

Jonathan Lane Larson

Student's signature

29 April 2014

Date

ASSESSING AND MITIGATING LAWN INSECTICIDE HAZARDS TO BEES AND  
OTHER BENEFICIAL INVERTEBRATES

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## TABLE OF CONTENTS

Acknowledgements.....	iii
List of Tables.....	viii
List of Figures.....	x
Chapter One: Introduction.....	1
Chapter Two: Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass.....	6
Introduction.....	6
Materials and Methods.....	8
Impacts on predators and predation, August–September 2009.....	9
Effects on earthworm and soil microarthropods, 2009.....	11
Impacts on predators, predation, decomposers and decomposition, 2010.....	12
Effects on ant mounding and earthworm casting, 2010.....	14
Statistical analyses.....	16
Results.....	16
Impacts on predators and predation, late summer 2009.....	16
Effects on earthworm and soil microarthropods, 2009.....	18
Impacts on predators, predation, decomposers and decomposition, 2010.....	21
Effects on ant mounding and earthworm casting.....	24
Discussion.....	29



Chapter Three: Impacts of a neonicotinoid, neonicotinoid–pyrethroid premix, and anthranilic diamide insecticide on four species of turf inhabiting beneficial insects.....	35
Introduction.....	35
Materials and Methods.....	37
Insecticides.....	37
Acute intoxication of a ground beetle by insecticide-treated food.....	37
Residual effects on parasitism of root-feeding grubs.....	39
Contact toxicity to an encyrtid parasitoid of black cutworm .....	41
Acute effects on bumble bee colonies exposed to residues on flowering weeds.....	42
Results.....	44
Acute intoxication of a ground beetle by insecticide-treated food.....	44
Residual effects on parasitism of root-feeding grubs.....	44
Contact toxicity to an encyrtid parasitoid of black cutworm.....	47
Acute effects on bumble bee colonies exposed to residues on flowering weeds.....	47
Discussion.....	50
Chapter Four: Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns.....	54
Introduction.....	54
Materials and Methods.....	57
Insecticide impacts on foraging, colony health and queen production.....	57

Acute effects of exposure to insecticide residues before and after mowing.....	59
Avoidance.....	60
Statistical analyses.....	61
Results.....	62
Discussion.....	67
Chapter Five: Mowing mitigates bioactivity of neonicotinoid insecticides in nectar of flowering lawn weeds and turfgrass guttation.....	76
Introduction.....	76
Materials and methods.....	78
Measurements of residues levels in clover nectar.....	78
Measurement of imidacloprid transfer into turf guttation droplets.....	80
Toxicity trials with <i>Orius insidiosus</i> .....	82
Statistical analyses.....	83
Results.....	83
Nectar analyses and toxicity to <i>Orius insidiosus</i> .....	83
Guttation analysis and <i>Orius</i> feeding assay.....	86
Discussion.....	86
Chapter Six: Pollinator diversity on dandelions and white clover in urban and suburban lawns.....	93
Introduction.....	93
Materials and methods.....	95
Pollinator assemblages of flowering dandelion and white clover in suburban lawns.....	95

Pollinator assemblages of white clover in lawns across an urban-rural gradient.....	96
Data analyses.....	98
Results.....	98
2011 clover-dandelion pollinator collections.....	98
2012 clover-dandelion collections.....	99
Pollinator assemblages of white clover in lawns across an urban-rural gradient.....	104
Discussion.....	109
Chapter Seven: Summary and implications.....	112
References.....	120
VITA.....	135

## LIST OF TABLES

Table 2.1.	Predation on cohorts of black cutworm, <i>A. ipsilon</i> , eggs exposed in plots in a golf course rough at 1 or 3 weeks after treatment with representatives of different insecticide classes in August 2009.....	19
Table 2.2.	Predation on sentinel black cutworm eggs exposed for 24 h and Japanese beetle eggs buried for 7 days in plots on a golf rough at specified weeks after treatment (WAT) with representatives of different insecticide classes, 2010.....	25
Table 2.3.	Decomposition of tall fescue grass clippings in buried litter bags after plots on a golf course rough were treated with insecticides representing different chemical classes in May 2010.....	27
Table 2.4.	Relative effectiveness of chlorantraniliprole versus bifenthrin application in reducing mounding activity by the ant <i>Lasius neoniger</i> on creeping bentgrass golf course tees.....	28
Table 2.5.	Mean ( $\pm$ SE) earthworm casts per m <sup>2</sup> at indicated weeks after treating plots on a soil-based putting green, and relative earthworm abundance at the end of the 5 week trial.....	30
Table 3.1.	Number of ground beetle ( <i>H. pennsylvanicus</i> ) adults, out of 30, displaying progressive poisoning symptoms during 48 h access to insecticide-contaminated food.....	45
Table 3.2.	<i>Tiphia vernalis</i> parasitism of Japanese beetle grubs in turf cores treated with a neonicotinoid, anthranilic diamide, or premix insecticide 48 h before introducing the wasps.....	46
Table 4.1.	Condition of <i>Bombus impatiens</i> colonies that had been exposed to insecticide-treated turf with flowering white clover for 6 days, after which they were moved to an insecticide-free site to openly forage for 6 weeks before this evaluation.....	66
Table 4.2.	Condition of <i>Bombus impatiens</i> colonies that were evaluated after being exposed to insecticide-treated flowering white clover for 2 wk.....	68
Table 4.3.	Absence of acute adverse effects on <i>Bombus impatiens</i> colonies after 2 weeks' exposure to turf with flowering white clover that had bloomed after the sward was mown to remove flowers present at the time of treatment.....	69

Table 5.1.	Insecticide residue levels found in two trials (spring and fall) of white clover nectar directly contaminated with either imidacloprid or clothianidin and levels of the same insecticides in new flowers from the same plots after mowing.....	84
Table 5.2.	Imidacloprid residue levels found in guttation droplets collected from creeping bentgrass ( <i>Agrostis stolonifera</i> ) at one and three weeks after treatment.....	87
Table 6.1.	Total numbers and the percentage of overall collection for each pollinator species captured on dandelion and clover flowers in 2011, categorized by tribe or family.....	100-101
Table 6.2.	Total numbers and the percentage of overall collection for each pollinator species captured on dandelion and clover flowers in 2012, categorized by tribe or family.....	103
Table 6.3.	Total pollinators collected from flowering white clover at 18 lawn sites in urban, suburban, or periurban settings based on amount of impervious surface area within a 200-m radius.....	106

## LIST OF FIGURES

Figure 2.1.	Pitfall captures of predatory invertebrates in golf course roughs during 1 week intervals before and after plots were treated with representatives of different insecticide classes on 20 August 2009.....	17
Figure 2.2.	Earthworm (mainly <i>Apporectodea</i> spp.) numbers and biomass in fairway-height creeping bentgrass treated with representatives of different insecticide classes on 1 October 2009.....	20
Figure 2.3.	Abundance of soil microarthropods in samples fairway-height creeping bentgrass treated with representatives of different insecticide classes on 1 October 2009.....	22
Figure 2.4.	Pitfall captures of predatory invertebrates in golf course roughs during 3 day trapping periods before and after plots were treated with representatives of different insecticide classes on 14 May 2010.....	23
Figure 2.5.	Abundance of soil microarthropods in samples from a golf course treated with representatives of different insecticide classes on 14 May 2010.....	26
Figure 3.1.	Response of cohorts of 30–50 adult <i>Copidosoma bakeri</i> exposed to dry residues of turfgrass insecticides showing knockdown (1 h) and mortality (3 h) from reduced rates of clothianidin or a clothianidin - bifenthrin premix compared to the untreated control (UTC), and absence of intoxication from chlorantraniliprole (Chlor).....	48
Figure 3.2.	Condition of <i>Bombus impatiens</i> colonies sacrificed and examined after 2 weeks' foraging on flowering white clover blooms that had been sprayed with clothianidin or a clothianidin ± bifenthrin premix when the weedy turf was treated at label rates for grub control.....	49
Figure 4.1.	Foraging and dead workers during exposure to treated turf. Mean (±SE) numbers of (A) bees foraging in enclosures during two mid-afternoon inspections on the 5th and 6th days, and (B) dead non-callow workers observed in hives on the 6th day of exposure of bumble bee colonies to weedy lawn turf with residues of a neonicotinoid (clothianidin) or anthranilic diamide (chlorantraniliprole) applied at label rates.....	63
Figure 4.2.	Colony weight change following exposure to treated turf. Mean (+ SE) weight change (g) of <i>Bombus impatiens</i> colonies (10 per treatment) after foraging 6 days on insecticide-treated lawn turf with white clover and then	

	being moved to an insecticide free site to openly forage for another 6 weeks .....	64
Figure 4.3.	Queen production following exposure to treated turf. Mean (+ SE) numbers of queens produced by <i>Bombus impatiens</i> colonies that foraged for 6 days on insecticide-treated lawn turf with white clover and then were moved to an insecticide-free site to openly forage another 6 weeks.....	65
Figure 4.4.	Non-avoidance of treated turf by bees. Bumble bees and honey bees did not discriminate between weedy lawn turf with or without non-irrigated insecticide residues ( $F_{2,8} = 0.02$ , $P = 0.98$ ). Plots were treated at label rate with residues left on the surface (not watered in) and a walk-through count of foragers on the intermixed white clover was taken on seven successive days. Data shown are mean (+ SE) totals of both types of bees.....	70
Figure 5.1.	<i>Orius</i> feeding trials using nectar from white clover flowers.....	85
Figure 5.2.	<i>Orius</i> feeding trials using guttation droplets collected from creeping bentgrass.....	88
Figure 6.1.	<i>A. mellifera</i> , <i>Bombus</i> spp., solitary bees, and syrphid flies collected from flowering dandelion in the spring and white clover in both spring and late summer.....	102
Figure 6.2.	Relative abundance of honey bees ( <i>A. mellifera</i> ), bumble bees ( <i>Bombus</i> spp.), and solitary bees (Andrenidae, Halictidae, and Megachilidae) in 50-pollinator samples collected from flowering white clover in 18 lawn sites classified as urban, suburban, or periurban-rural.....	105
Figure 6.3.	Number of <i>A. mellifera</i> or <i>Bombus</i> spp. in 50-pollinator samples from flowering white clover in 18 lawns surrounded by differing amounts of impervious surface within a 200 m radius.....	108

## CHAPTER ONE

### Introduction

In the United States there are about 164,000 km<sup>2</sup> of turfgrass cultivated for use on golf courses, sports fields, and home and commercial lawns (Milesi et al. 2005). Healthy turf offers environmental benefits including cooling ground areas, improving drainage, filtration of rainfall, and carbon sequestration (Beard & Green 1994, Bandaranayake et al. 2003). Dense, uniform turf increases the playability and safety of golf courses and sports fields and can improve the aesthetics and value of homes (Beard and Green 1994).

In Kentucky, which is part of the transitional climatic zone, several different kinds of insect pests can cause extensive damage to turfgrass (Potter 1998). Larvae of the Japanese beetle, *Popillia japonica* Newman, southern masked chafer *Cyclocephala lurida* Bland, northern masked chafer, *Cyclocephala borealis* Arrow, and black turfgrass ataenius, *Ataenius spretulus* Haldeman, chew off the roots and cause symptoms such as yellowing, wilting, and death of irregular large patches of turf. Lepidopteran pest species include black cutworms, *Agrotis ipsilon* Hufnagel, armyworms and fall armyworm, *Pseudaletia* spp and *Spodoptera frugiperda* Smith, and sod webworms (various species of Pyralidae). These feed above ground and cause damage to leaf blades and stems causing thin and dying stands of turf with pock-marked, uneven playing surfaces. Turfgrass managers typically apply insecticides in the spring as a preventive measure before pest populations, in particular white grubs, can become established (Blaine et al. 2012, Held and Potter 2012). However, use of insecticides on turfgrass is controversial due to concerns over ground water contamination, real or perceived hazard to people,



pets, and wildlife, and other side effects. These concerns have led to bans on insecticide use on turf in places such as Canada (Sandberg and Foster 2007) and to several global initiatives aimed at moving turf care towards more environmentally sustainable practices (Termann 1997, Gange et al. 2003, USGA 2006).

While there are many types of insecticides registered for use on turf, three groups in particular dominate the market because they control a broad range of pests and have relatively long residual activity. These include premix combination products containing a pyrethroid and a neonicotinoid, the neonicotinoids, and recently the anthranilic diamides.

To save fuel, time, and labor some turf managers may try to preventively control foliage and root-feeding pests with a single insecticide application (Houseworth 2009, Buss 2012). This multiple-targeting, typically entails applying a tank mix or premix formulation with two or more active ingredients or a product with one active ingredient with broad activity (Buss 2012). Premixes labelled for use in turf typically contain two neurotoxicants; a pyrethroid that binds to foliage and thatch and controls surface-active pests such as caterpillars and chinch bugs, plus a neonicotinoid that, when watered-in, controls soil dwelling pests such as scarab grubs and weevil larvae. Another advertised benefit offered by premixes is the potential for increased pest suppression due to target-site-based synergy (Elbert et al. 2008, Houseworth 2009, Buss 2012). However, if synergism occurs, premixes may also be more toxic to beneficial invertebrates than their individual components alone (Gill et al. 2012).

Neonicotinoids are systemic, neurotoxic insecticides. They are agonists of nicotinic acetylcholine receptors in insects (Tomizawa and Casida 2003). In turf they are

applied as sprays or granules in spring, and watered in by irrigation or rainfall. They typically provide several months' residual control of root-feeding beetle grubs. Although neonicotinoids have relatively low toxicity to humans, there are potential hazards to non-target invertebrates from contact toxicity or consumption of contaminated food (Kunkel et al. 2001, Rogers and Potter 2003, Capowiez and Berard 2006). Neonicotinoids are translocated acropetally in xylem and can control some stem- and foliage-feeding pests (Held and Potter 2012). However, translocation from soil could also occur in nectar, pollen, or guttation of treated plants, and be ingested by bees or other beneficial insects (Shawki et al. 2005, Thompson 2010, Cresswell 2011).

Anthranilic diamides are a relatively new class of insecticides that activate insect ryanodine receptors by stimulating release of calcium stores from muscle cells causing lethal paralysis in sensitive species (Cordova et al. 2006). Chlorantraniliprole, the first anthranilic diamide lawn insecticide, has low vertebrate toxicity, low use rates, 3–5 month residual activity in soil and is relatively less toxic to non-target terrestrial and aquatic invertebrates (Cordova et al. 2006, Brugger et al. 2010, Barbee et al 2010). Compared to neonicotinoids, it has similar efficacy against root-feeding scarab grubs and weevil larvae, better activity against caterpillar pests, but is less active against chinch bugs (Held and Potter 2012). Compared to premix formulations it provides the same multiple target control but with one active ingredient. I sought to define the field effects this new insecticide class might have on non-target invertebrates.

Turf supports many species of beneficial invertebrates including natural enemies that help to suppress pest populations (Cockfield and Potter 1984; Bixby-Brosi and Potter 2012a), decomposers that degrade and improve soil tilth (Potter et al. 1990; Potter 1993;

Peck 2009), and pollinators that forage on flowering lawn weeds (Shepherd and Tepedino 2000). Turf industry initiatives toward sustainability (Racke 2000; Gange et al. 2003; Dobson 2005) are facilitated by using insecticides that conserve populations and the services provided by beneficial invertebrates (Potter 1993; Bixby-Brosi and Potter 2012a). Assessments of lawn insecticide hazards to these beneficial species should use trials that simulate realistic exposure dosages and scenarios (Stark et al. 1995).

One of the main themes of this dissertation is to help inform industry-wide sustainability initiatives. To that end it reports the results of several experiments aimed at clarifying the effects of three types of insecticides, neonicotinoids, premix formulations, and anthranilic diamides. In particular, research was focused on three guilds of beneficial invertebrates; the natural enemies, the decomposers, and the pollinators. A commonly used representative from each insecticide group was tested for lethal and sub-lethal impacts after realistic exposure scenarios.

This dissertation is organized into seven distinct chapters. Chapter One is a general introduction. Chapters Two, Three, Four, Five, and Six are in publication format, each with its own introduction, methods, results, and discussion. More specifically, Chapter Two reports a two year study concerning the effects of turf grass insecticides on predators, predation rates, decomposers, and rates of decomposition in the field settings and implications for turfgrass managers. Chapter Three concerns the impacts of four different insecticides used in turf on four specific non-target beneficial invertebrates. Chapter Four focuses on the potential hazards of lawn insecticides to pollinating insects when these products are oversprayed onto flowering weeds and possible means to mitigate risks. Chapter Five documents the systemic movement of lawn neonicotinoid

insecticide residues from soil into the nectar of blooming white clover and the guttation fluid of turfgrass and assesses the bioactivity associated with this systemic movement.

Chapter Six reports the results of collections of pollinating insects made on two flowering lawn weeds, white clover and dandelion, and assesses the impact that hardscape such as pavement and concrete can have on urban pollinating insect communities. Chapter Seven is an interpretive summary. References are listed at the end of the dissertation.

## **CHAPTER TWO**

### **Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass**

#### **Introduction**

Chlorantraniliprole, the first anthranilic diamide insecticide labeled for turf, combines low use rates, 3–5 month residual activity in soil and very low toxicity to vertebrates (Cordova et al. 2006). Anthranilic diamides have a novel, specific mode of action, activating ryanodine receptors via stimulation of the release of calcium stores from the sarcoplasmic reticulum of muscle cells and causing impaired regulation and lethal paralysis in sensitive species (Cordova et al. 2006, Lahm et al. 2007).

Chlorantraniliprole has strong differential selectivity towards insect ryanodine receptors, which accounts for its low mammalian toxicity and favorable environmental profile and its receiving reduced risk status from the US Environmental Protection Agency for use on turf. It also has low intrinsic toxicity to honey bees, bumblebees and parasitoids (Brugger et al. 2009, Gradish et al. 2010) and low potential of harmful systemic exposure via pollen and nectar (Dinter et al. 2009). Chlorantraniliprole is effective against the entire spectrum of turf-damaging scarab grubs, as well as billbug and other weevil larvae, grass-feeding caterpillars and invasive crane flies (Held and Potter 2012).

Lawns, golf courses, and sports fields are inhabited by a diverse community of beneficial invertebrates that contribute to pest suppression, decomposition of clippings and thatch, nutrient recycling and good soil tilth (Potter 1993, Bixby-Brosi and Potter 2012). Although all classes of turf insecticides registered since 1993 have relatively low

mammalian and avian toxicity (Potter 2005, Held and Potter 2012) some (e.g. pyrethroids, neonicotinoids) are acutely toxic to pollinators (Gels et al. 2002) and they may also impact natural enemies or earthworms enough to temporarily disrupt the positive ecosystem services of these organisms (Kunkel et al. 1999, Rogers and Potter 2003, Capowiez and Berard 2006). Conservation of non-target species, including beneficial invertebrates, is consistent with industry-wide initiatives for environmental stewardship and more sustainable management of golf courses, (Terman 1997, Gange et al. 2003, Dobson 2005, USGA 2011) lawns, and landscapes (National Wildlife Federation: <http://www.nwf.org/Get-Outside/Outdoor-Activities/Garden-for-Wildlife.aspx>). Target-selective insecticides offer potential for integrating chemical and conservation biological control (Potter 1993, Potter 2005, Held and Potter 2012, Bixby-Brosi and Potter 2012).

Post-patent marketing of mixtures of active ingredients is another industry trend. Typically, such products contain a pyrethroid, which binds to foliage and thatch and controls surface-active pests, and a neonicotinoid, which is watered in to control root-feeding pests as well as certain stem feeders (e.g., billbug larvae) and foliage feeders by systemic action. Purported benefits of premix combination products include broader, faster or longer-lasting control, savings in time, fuel and labor by reducing the number of applications and potential for target-site-based synergy (Houseworth 2009). The latter concept is controversial, although there is some laboratory evidence for greater than additive neurophysiological effects of imidacloprid + bifenthrin on mole crickets (Kostromytska et al. 2011). Such synergism, if it occurs, would logically also extend to exposure of non-target invertebrate species.

This study tested the hypothesis that chlorantraniliprole, a representative anthranilic diamide, is less disruptive to beneficial invertebrates and associated ecosystem services than are representatives of other widely used classes of turf insecticides. Stark et al. (1995) emphasized that tests used to evaluate and predict pesticide hazards to beneficial species should be designed to simulate field conditions realistically, so the trials were done in golf course settings with rates and application methods similar to those used by turf managers. Predatory ants and earthworms, while beneficial in most turf settings (Potter et al. 1990, Potter et al. 1994, Lopez and Potter 2000) can become important pests when their mounds or casts disrupt the smoothness and uniformity of short-mowed playing surfaces (Maier and Potter 2005a, Potter et al. 2010). Therefore, an ancillary hypothesis is that, because of its selectivity, the anthranilic diamide will also provide less suppression of these problems than occurs as a side effect of the use of certain other chemistries.

### **Materials and Methods**

The main trials comparing insecticide impacts on non-target invertebrates, predation on pest eggs and decomposition were conducted in untreated roughs at two central Kentucky golf courses. Although preventive treatments for scarab and weevil larvae are typically applied in spring, each of the insecticide classes may also be applied in late summer to control foliage-feeding pests, mound-building ants and scarab grubs, and sometimes for off-label suppression of earthworms and associated casts. Additional studies to evaluate impacts on populations of earthworms and other invertebrate decomposers, earthworm casting and ant mounding were conducted at other sites as noted below.

## Impacts on predators and predation, August–September 2009

This trial was replicated across Kentucky bluegrass, *Poa pratensis* L., roughs at Idle Hour Golf Course, Lexington, Kentucky. Plots ( $6 \times 6$  m, with 2 m untreated borders) were arranged as a randomized complete block with six replicates and four treatments. Nine permanent pitfall traps were set in each plot, one in the center and the two others at 1 and 2 m from the center along diagonal transects toward each plot corner. Traps were plastic centrifuge tubes (50 mL, 3 cm diameter opening) inserted into cored-out holes. Each trap received 20 mL of ethylene glycol as a killing agent and preservative. Traps were operated for 1 week before treatment, and then the samples were collected and traps were removed before applying and watering in the insecticides.

The four treatments were chlorantraniliprole (Acelepryn, 18.4% active ingredient [AI]; Dupont, Wilmington, DE), clothianidin (Arena 50 WDG; Valent, Walnut Creek, CA), a combination product containing 24.7% clothianidin and 12.3% bifenthrin (Aloft; Arysta, Cary, NC), plus the untreated check. Chlorantraniliprole was applied at its high label rate for scarab grub control ( $0.23 \text{ kg AI ha}^{-1}$ ); the neonicotinoid and neonicotinoid–pyrethroid premix were applied at their single listed label rate for grubs, corresponding to  $0.45 \text{ kg AI ha}^{-1}$  clothianidin.

The applications were made with a portable CO<sub>2</sub> spray tank (R and D Sprayers, Opelousas, LA) equipped with a 1.8 m handheld boom with four Spraying System 8004 Tee Jet nozzles (Spraying Systems, Wheaton, IL) that delivered a pressure of  $2109 \text{ g cm}^{-2}$ . Spray volume was  $468 \text{ L ha}^{-1}$ , applied by making two passes in opposite directions over each plot. Separate spray bottles were used for each treatment. Treatments were



applied on 20 August and were followed within 1 h by about 1.5 cm of irrigation from the golf course sprinkler system. Pitfall traps were replaced as soon as the residues had dried.

Traps were emptied weekly for 4 weeks after treatment. Within-plot samples were consolidated, and fresh preservative was added before replacing the traps. Weekly samples were sorted, and specimens belonging to predominantly predatory taxa, including Araneae (spiders), Formicidae (ants), Staphylinidae (rove beetles) and Carabidae (ground beetles), were counted.

Effects on predatory activity were evaluated by exposing groups of black cutworm, *Agrotis ipsilon* (Hufnagel), eggs in the plots. The eggs, from females in a lab colony, had been deposited on white muslin that was cut into pieces (2 cm<sup>2</sup>), each with 20 one-day-old eggs. The cloth pieces with 20 eggs were taped 6 cm above the pointed end of wooden garden stakes (25.4 cm). Two such 'egg sticks' were placed in each plot at 1 and 3 weeks after treatment (i.e. 40 eggs per plot per date). The sticks were placed 1 m to either side of the central pitfall trap, equidistant between the traps on the diagonals, and pushed into the soil so that the cloth was flush with the base of the grass plants. Egg sticks were left in plots for 24 h and then pulled out and examined under a dissecting scope to determine the number of eggs that were missing or had otherwise been preyed upon. Eight additional sticks (160 total eggs) with sticky (Tanglefoot, Grand Rapids, MI) barriers above and below the cloth were placed in the border areas between plots during each trial as egg hatch controls.

## Effects on earthworm and soil microarthropods, 2009

This trial was conducted in a stand of ‘Penncross’ creeping bentgrass, *Agrostis stolonifera* L., on a Maury silt loam (fine, mixed, mesic Typic Paleudalf; pH = 6.0) at the University of Kentucky's AJ Powell, Jr, Turfgrass Research Center (UKTRC), Spindletop Farm, near Lexington, KY. The turf, managed as a golf course fairway, was mowed 3 times per week at 1.6 cm and irrigated as necessary to prevent drought stress, with fertilizer (urea: 46-0-0) applied in September, October and November at 0.48 kg N 100 m<sup>-1</sup> per application. Plots (1.5 × 1.5 m, with 0.5 m untreated borders) were arranged in a randomized complete block with six replicates. The five treatments were the same as those used in the previous trial, plus the carbamate insecticide carbaryl (Sevin SL, 43% AI, 9.16 kg AI ha<sup>-1</sup>; Bayer, Research Triangle Park, NC), which was included as an earthworm-toxic standard (Potter et al. 1994). The insecticides were applied on 1 October and watered in as described above.

Earthworms were sampled on 8 and 29 October, 1 and 3 weeks after treatment, by taking four soil cores (15.2 cm diameter, 15 cm deep) from each plot with a golf course cup cutter. The cores were broken apart by hand and examined in the field, and earthworms (mostly *Apporectodea* spp.) were collected into plastic bags with wet paper towels, brought to the lab, counted and weighed. The few nightcrawlers (*Lumbricus terrestris* L.) that were collected were excluded from analyses owing to their substantially greater individual mass.

The impacts of the insecticides on soil microarthropods were assessed by taking two soil cores (5.1 cm diameter, 7.6 cm deep) from each treated plot concurrently with

the earthworm sampling. The cores were consolidated within the plot, quartered using a hacksaw, lightly crumbled by hand and placed in Tullgren funnels (Burkhardt Agronomic Instruments, Uxbridge, Middlesex, UK) equipped with 25 W bulbs for 48 h. Samples were stored in 70% ethanol until sorted. The predominant taxa, oribatid and mesostigmatid mites and Collembola, were counted with a dissecting microscope.

### **Impacts on predators, predation, decomposers and decomposition, 2010**

The trials described in 2009 were repeated in 2010, with the following modifications. The study site was an untreated Kentucky bluegrass rough at the Lexington Country Club, Lexington, KY. Plots ( $10 \times 10$  m, with 2 m untreated borders) were arranged in a randomized complete block with six replicates and five treatments. The plots were treated at label rate with the same chemicals as in 2009, with the addition of bifenthrin (Talstar Select; 7.9% bifenthrin; FMC, Philadelphia, PA) at the label rate for surface-feeding pests ( $0.064 \text{ kg AI ha}^{-1}$ ) so that both active ingredients in the combination product were also individually represented. The insecticides were applied as above on 14 May, followed by irrigation (1.5 cm)  $\leq 1$  h after treatment. In 2010, larger pitfall traps (473 mL plastic cups, 10 cm diameter; Solo, Lake Forest, IL) were used for better capture of ground beetles and other relatively large predators. Five traps were arranged in each plot, one central and the others at 1.5 m from the center diagonally towards each corner.

Traps were operated continuously for 3 days immediately before treatment, removed when the insecticides were applied and then replaced for additional 3 day trapping periods at 1, 3, 6 and 12 weeks after treatment. Within-plot samples were

consolidated and sorted as before. Specimens of Formicidae, Staphylinidae, Carabidae and spiders, the four most abundant predominantly predatory taxa, were counted.

Impacts on predation were assessed by exposing cohorts of black cutworm and Japanese beetle, *Popillia japonica* Newman, eggs in treated and untreated plots. Egg sticks, each with 15 cutworm eggs, were prepared as described earlier. Two such sticks were placed in each plot, along the diagonals as in 2009, at 1, 3, 6 and 9 weeks after treatment (i.e. 30 eggs exposed per plot per date), left in the field for 48 h and then pulled out and examined. Hatching controls were included as above. Field-caught Japanese beetles were held in the laboratory in bins of moist soil with linden (*Tilia* sp.) leaves as food. Eggs (<2 days old) were collected from the soil. Ten eggs were placed in each of 90 petri dish bottoms (5 cm diameter) with a small amount of moist soil. Three dishes were implanted at 1 m distances around the central pitfall trap in each plot. This was done by removing three turf cores with a golf course cup cutter, cutting a slit about 2 cm below the thatch–soil interface into the side of each hole and sliding the open dish into the slit, after which the core was replaced. The dishes with eggs were implanted on 19 July, and remaining eggs were counted after 1 week.

Impacts of the insecticides on soil microarthropods were assessed by taking two soil cores (5.1 cm diameter, 7.6 cm deep) from each treated plot 3 weeks after application. Cores were consolidated by plot and quartered using a hacksaw. Specimens were extracted using Tullgren funnels as described earlier and stored in 70% ethanol, and the predominant taxa, including oribatid and mesostigmatid mites and Collembola, were counted.

Litter bags with grass clippings were buried to assess insecticide impacts on rates of decomposition. Pouches ( $7.5 \times 10.5$  cm) were constructed of plastic mesh screening ( $2 \times 2$  mm openings) with heat-sealed seams. A pre-weighed (3 g) sample of oven-dried non-endophytic tall fescue (*Festuca arundinacea* Schreb.) clippings (about 4 cm long) was placed in each bag. Two such bags were buried just under the soil–thatch interface (3–4 cm deep) on 14 May 2010, the morning before the insecticides were applied. This was done by cutting a shallow diagonal slit with a flat-blade spade, inserting the bags and tamping down the turf and soil. Each litter bag had a numbered aluminum tag so that it could be found using a metal detector. The implanted bags were left in place during the spray applications, and then separate sets were recovered at 2 or 4 months after treatment. Remaining grass clippings were oven dried and weighed.

### **Effects on ant mounding and earthworm casting, 2010**

Trials were conducted to test the hypothesis that selective insecticides might result in less suppression of nuisance ant mounds and earthworm casts than occurs as a side effect when broader-spectrum chemistries are applied. The first two trials compared suppression of mounding by the ant *Lasius neoniger* (Emery) by chlorantraniliprole versus bifenthrin, a standard for controlling cutworms and other surface-active insect pests on golf courses (Held and Potter 2012). Eighteen plots ( $3.7 \times 3.7$  m) were marked on creeping bentgrass tees with abundant ant mounds at Champion Trace Golf Course, Nicholasville, KY. Most plots were on separate tees; a few large tees had two plots separated by at least 12 m. Treatments, including the untreated checks, were blocked by pretreatment counts taken on 10 June 2010 by centering a PVC pipe frame ( $2.44 \times 2.44$  m) in each plot and counting the total mounds within the frame. Plots were treated on 11

June at the same rates and in the same manner as those described in Section 2.3. The tees were irrigated (1.5 cm) from the golf course sprinkler system on the night of 11–12 June and maintained under the normal mowing and watering regime. No additional insecticides were applied. Treatments were evaluated on 17 June and 1 July by placing the aforementioned frame in the central portion of each plot and counting the total number of ant mounds therein. The trial was repeated on the same golf course in 2011, using different plots but the same methods, treatments and rates as in 2010. The insecticides were applied on 20 April, and mounds were counted 1 and 3 weeks later.

The final trial compared effects of chlorantraniliprole, clothianidin, the clothianidin + bifenthrin combination and carbaryl on earthworm casting activity. The study site was a stand ( $29 \times 29$  m) of creeping bentgrass and annual bluegrass, *Poa annua* L. (species distribution by visual estimation 75 and 25% respectively) established in 1978 and managed as a push-up golf putting green at the UKTRC. The soil was homogeneous and original, and no sand topdressing had been applied. The turf was mowed 5 times per week (4.0 mm height of cut) and irrigated from a permanent sprinkler system to prevent visible stress. The site had a history of high numbers of earthworms (mostly *Apporectodea* spp.), and there was active casting in the weeks before and during the trial. Plots ( $1.5 \times 1.5$  m, six replicates, with 0.5 m untreated borders) were marked, and casts were counted in the inner  $1 \text{ m}^2$  of each plot on 16 April. Treatments were blocked by these counts and applied on the same day. Insecticides and rates were the same as in 2010 trials. Earthworm casts in each plot were counted on 3 and 19 May. The stand was mowed every 2–3 days, but fresh casts were allowed to accumulate for 1 day and night before each sample date. On 25 May, all plots were treated with tea seed pellets (1.83 kg

100 m<sup>-2</sup>), a saponin-rich byproduct of Camellia oil manufacture that irritates and causes earthworms to come to the surface (Potter et al. 2010). The tea seed treatment was watered in (1.5 cm of irrigation), and earthworms surfacing in each plot were counted.

### **Statistical analyses**

Data were analyzed within each trial by two-way analysis of variance (ANOVA) with means separation by Fisher's protected least significant difference (LSD) test ( $P = 0.05$ ) when the ANOVA indicated a significant ( $P < 0.05$ ) treatment effect. Log or square-root transformations were applied when needed to correct for heterogeneity of variance in count data, whereas the arcsine square-root transformation was applied to percentages. Analyses were done with Statistix 8.0. Data are presented as original means  $\pm$  standard error (SE).

## **Results**

### **Impacts on predators and predation, late summer 2009**

The three most abundant taxa of predominantly predatory invertebrates captured in pitfall traps were Formicidae [mostly *L. neoniger* and *Solenopsis molesta* (Say)], Araneae (mostly Linyphiidae, Erigonidae and Lycosidae) and Staphylinidae [mostly *Platydracus mysticus* (Erichson), *Philonthus cognatus* Stephens and *Philonthus carbonarius* (Gyllenhal)]. Pretreatment counts did not differ for any of above groups ( $F_{3, 15} \leq 0.45$ ,  $P \geq 0.58$ ) (Figure 2.1), but there were significant post-treatment effects on ants ( $F_{3, 15} = 6.2, 6.6, 5.4$  and  $6.1$  at 1, 2, 3 and 4 weeks respectively;  $P < 0.01$ ) and spiders ( $F_{3, 15} = 3.2, 4.6$  and  $6.6$  at 1, 2, and 4 weeks respectively;  $P < 0.05$ ). Chlorantraniliprole did not significantly affect captures of any of the aforementioned groups compared with the

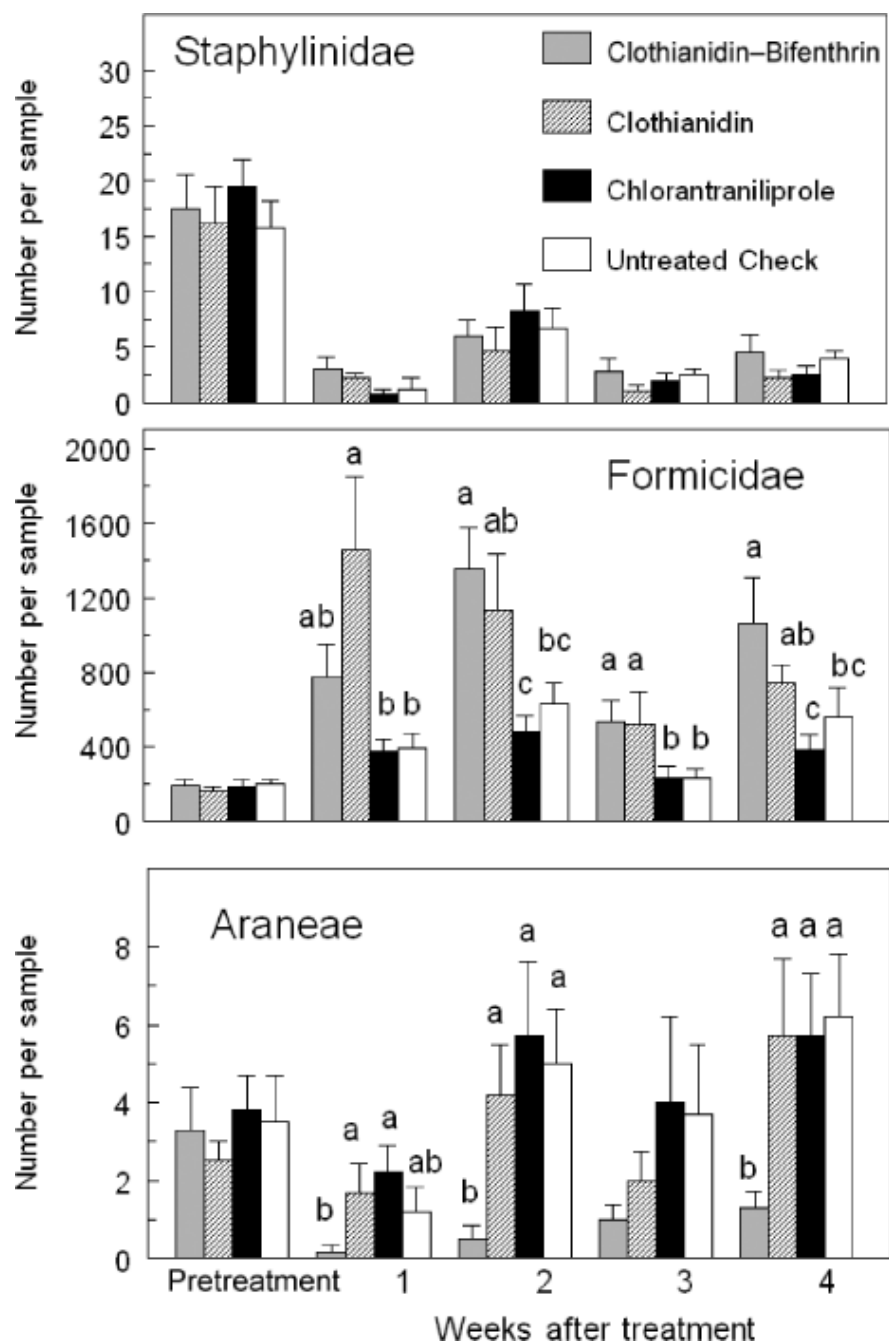


Figure 2.1. Pitfall captures of predatory invertebrates in golf course roughs during 1 week intervals before and after plots were treated with representatives of different insecticide classes on 20 August 2009. Data are means ( $\pm$  SE) per plot. Post-ANOVA results are shown above those sets of bars for which the within-date treatment effect was significant ( $P \leq 0.05$ ). Within these sample intervals, bars not marked with the same letter differ significantly (LSD,  $P < 0.05$ ).



untreated check (Figure 2.1). There were no treatment effects on staphylinids, but the clothianidin–bifenthrin combination suppressed spiders on two of the four sample dates (Figure 2.1). Ant captures were elevated, relative to the untreated check, on multiple sample dates after treating the turf with the combination insecticide or clothianidin alone (Figure 2.1). Too few carabids (ten total specimens) were captured for meaningful analysis, probably because of the small (3 cm) diameter of the pitfall traps used in 2009.

Predators consumed 73–81% of the sentinel black cutworm eggs in the untreated plots within 48 h (Table 2.1). Neither chlorantraniliprole nor clothianidin affected egg predation. In contrast, the combination insecticide significantly reduced egg predation by 37–47% relative to the untreated checks (Table 2.1). There was no loss of eggs from the hatch checks.

### **Effects on earthworm and soil microarthropods, 2009**

Earthworm numbers ( $F = 12.8, 14.7; P < 0.001$ ) and biomass ( $F_{4, 12} = 3.8, 4.9; P < 0.05$ ) were reduced by some treatments on both sample dates. Chlorantraniliprole had no apparent impact, but the clothianidin–bifenthrin combination had reduced earthworm abundance and biomass, by 33 and 49% respectively after 1 week, and by 35 and 50% after 3 weeks (Figure 2.2). Clothianidin alone reduced earthworm numbers and biomass by 32 and 39% respectively after 1 week. Carbaryl, the reference toxicant, had an even greater short-term impact (Figure 2.2).

Oribatid mites showed no response to the treatments on either sample date ( $F_{4, 12} \leq 0.85, P \geq 0.51$ ), but predatory mites ( $F_{4, 12} = 9.5, 5.5; P < 0.01$ ) and Collembola ( $F_{4, 12} = 7.8, 18.2; P < 0.001$ ) were significantly impacted at 1 week and for at least 3 weeks

Table 2.1 Predation on cohorts of black cutworm, *A. ipsilon*, eggs exposed in plots in a golf course rough at 1 or 3 weeks after treatment with representatives of different insecticide classes in August 2009

Treatment	Mean ( ± SE) percentage of eggs taken in 48 ha	
	1 week after treatment	3 weeks after treatment
Clothianidin–bifenthrin	45.8 ± 8.7 b	43.3 ± 8.9b
Clothianidin	67.5 ± 13.0 ab	77.5 ± 4.9a
Chlorantraniliprole	88.8 ± 6.3 a	71.3 ± 13.1a
Untreated check	72.5 ± 11.7 a	81.3 ± 9.3a

Two-way ANOVA. 1 week:  $F_{3, 15} = 4.15$ ;  $P = 0.025$ . 3 weeks:  $F_{3, 15} = 3.12$ ;  $P = 0.058$ . Within columns, means not followed by the same letter are significantly different (LSD,  $P < 0.05$ ).

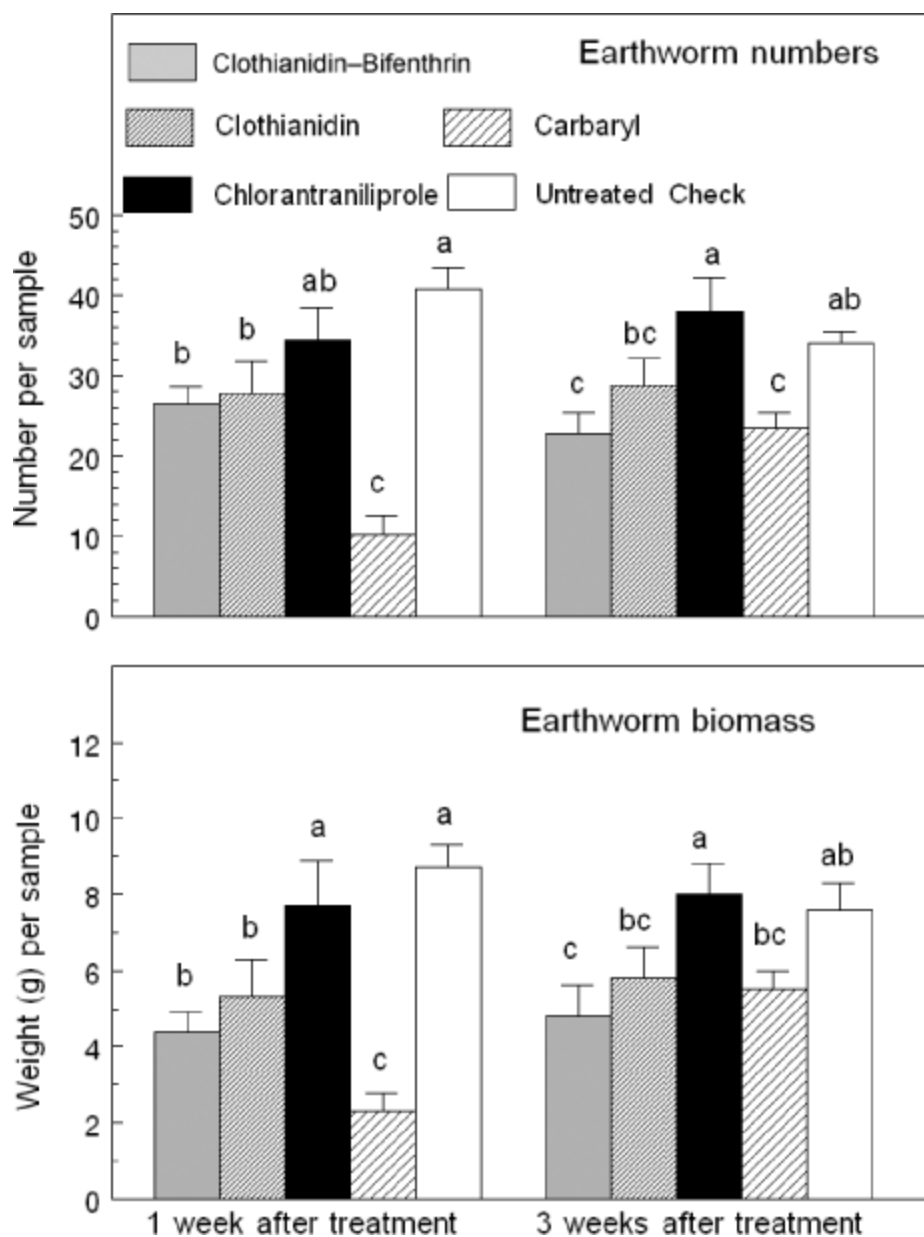


Figure 2.2. Earthworm (mainly *Apporectodea* spp.) numbers and biomass in fairway-height creeping bentgrass treated with representatives of different insecticide classes on 1 October 2009. Data are means ( $\pm$  SE) per plot. Post-ANOVA results are shown above those sets of bars for which the within-date treatment effect was significant ( $P \leq 0.05$ ). Within these sample intervals, bars not marked with the same letter differ significantly (LSD,  $P < 0.05$ ).

after treatment (Figure 2.3). Chlorantraniliprole had no measured effects, but clothianidin alone suppressed Collembola for at least 3 weeks, and the clothianidin–bifenthrin combination reduced numbers of predatory mites by 52–66% and the numbers of Collembola by 89–92% compared with populations in untreated turf. Carbaryl, too, suppressed both of these groups (Figure 2.3).

### **Impacts on predators, predation, decomposers and decomposition, 2010**

Chlorantraniliprole slightly suppressed pitfall captures of staphylinids (3 week samples) and spiders (3 and 6 weeks) but otherwise did not impact measures of predators compared with the untreated checks (Figure 2.4). Impacts of the other insecticides were greatest at 3 weeks after application ( $F_{3, 15} = 8.1, 12.0, 2.70$ ;  $P < 0.001, 0.001, 0.05$  for Staphylinidae, Araneae, and Carabidae, respectively). Bifenthrin and the combination insecticide reduced 1 and 3 week spider captures by  $> 80\%$  compared with the untreated check; the combination product also reduced 3 week captures of staphylinids and carabids by 67 and 83% respectively (Figure 2.4). By 12 weeks post-treatment, however, pitfall samples indicated no remaining differences in predator abundance and activity between treated and untreated plots ( $F_{4, 20} \leq 1.9$ ;  $P \geq 0.15$ ; data not shown). More carabids (predominantly *Harpalus pennsylvanicus* DeGeer and *Amara* spp.) were captured in 2010 than in 2009, probably because of the larger-sized pitfall traps that were used. Ant captures were not reduced by any treatment; rather, as in 2009, they tended to increase after clothianidin (1 week sample,  $F_{4, 20} = 2.46$ ,  $P = 0.08$ ) or the combination product (6 week sample,  $F_{4, 20} = 3.82$ ,  $P < 0.05$ ) was applied (Figure 2.4). Rates of predation on Japanese beetle eggs ranged from 65 to 83% and were not significantly

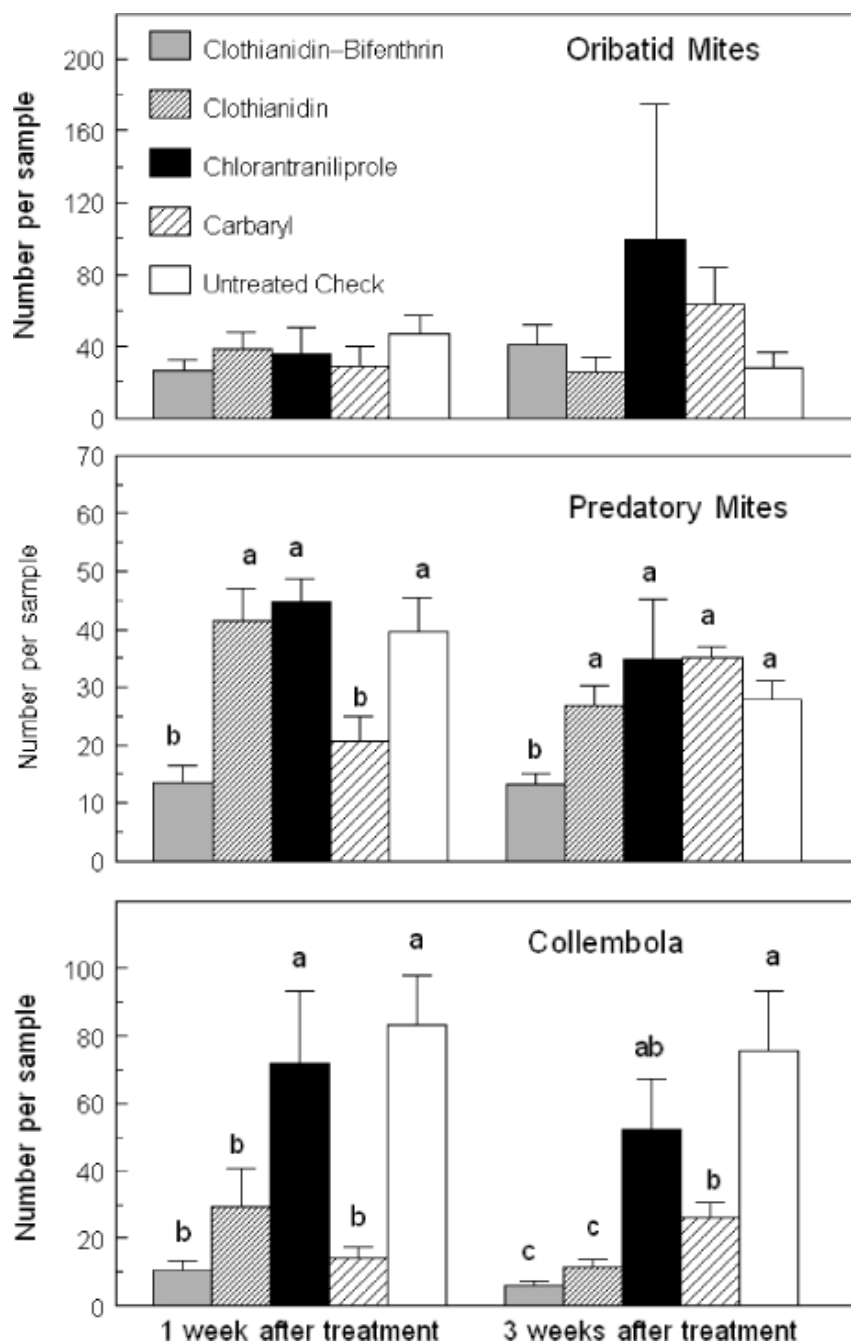


Figure 2.3. Abundance of soil microarthropods in samples fairway-height creeping bentgrass treated with representatives of different insecticide classes on 1 October 2009. Data are means ( $\pm$  SE) per plot. Post-ANOVA results are shown above those sets of bars for which the within-date treatment effect was significant ( $P \leq 0.05$ ). Within these sample intervals, bars not marked with the same letter differ significantly (LSD,  $P < 0.05$ ).

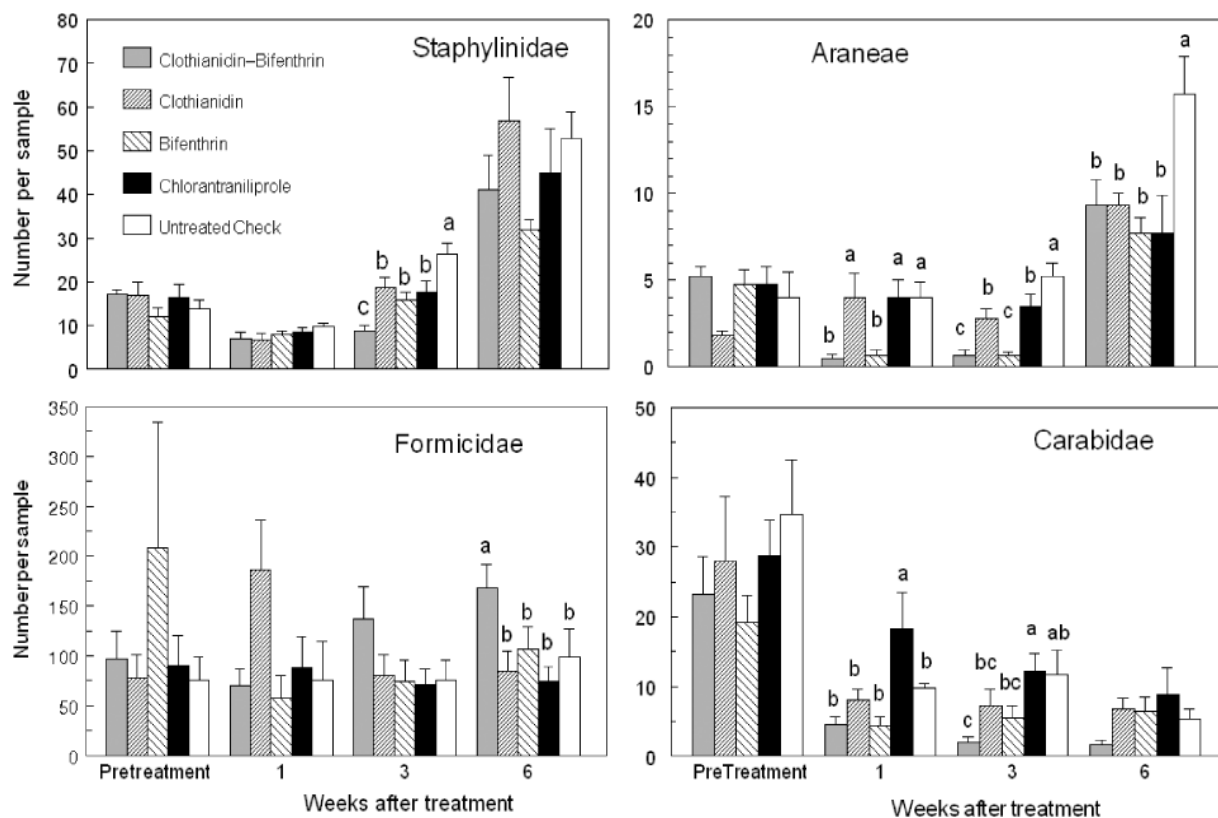


Figure 2.4. Pitfall captures of predatory invertebrates in golf course roughs during 3 day trapping periods before and after plots were treated with representatives of different insecticide classes on 14 May 2010. There were no significant treatment effects at the 12 week sample date (data not shown). Data are means ( $\pm$  SE) per plot. Post-ANOVA results are shown above those sets of bars for which the within-date treatment effect was significant ( $P \leq 0.05$ ). Within these sample intervals, bars not marked with the same letter differ significantly (LSD,  $P < 0.05$ ).

affected by any treatment (Table 2.2). Except for the combination insecticide, which reduced predation at 3 weeks after treatment, none of the other insecticides influenced the percentage of black cutworm eggs taken by predators (Table 2.2). There was no eclosion from the egg clusters that had been protected from predation as hatch checks.

None of the treatments significantly reduced the abundance of oribatid mites, predatory mites or Collembola relative to the untreated checks (Figure 2.5). Although there were significant differences among treatments for predatory mites after 3 weeks ( $F_{4, 20} = 2.83$ ,  $P = 0.05$ ) and for Collembola after 9 weeks ( $F_{4, 20} = 4.13$ ,  $P = 0.05$ ), they reflect the lesser abundance of these groups in plots where clothianidin–bifenthrin or carbaryl, as opposed to chlorantraniliprole, had been applied (Figure 2.5).

The clothianidin–bifenthrin combination, and to a lesser extent the clothianidin alone, retarded decomposition of grass clippings in the buried litter bags (Table 2.3). The dry weight of grass remaining in these treatments was 4.0 and 3.3-fold higher than in untreated plots after 2 months, and 9.7 and 6.9-fold higher respectively at 4 months after treatment.

### **Effects on ant mounding and earthworm casting**

In the 2010 trial, chlorantraniliprole had not reduced the numbers of ant (*L. neoniger*) mounds on treated golf course tees after 1 week, although there was some suppression after 3 weeks compared with the numbers of mounds on untreated tees (Table 2.4). Bifenthrin suppressed mounding activity by 90 and 65% at 1 and 3 weeks after treatment respectively (Table 2.4). Repetition of the trial in 2011 supported the

Table 2.2. Predation on sentinel black cutworm eggs exposed for 24 h and Japanese beetle eggs buried for 7 days in plots on a golf rough at specified weeks after treatment (WAT) with representatives of different insecticide classes, 2010

Treatment	Mean ( ± SE) percentage of eggs taken <sup>a</sup>			
	<i>A. ipsilon</i>		<i>P. japonica</i>	
	1 WAT	3 WAT	6 WAT	9 WAT
Clothianidin–bifenthrin	14.3 ± 4.6	27.7 ± 6.1	91.0 ± 2.2	73.0 ± 10.6
Clothianidin	40.0 ± 13.9	44.0 ± 11.4	95.0 ± 1.1	65.3 ± 7.1
Bifenthrin	37.6 ± 9.1	35.6 ± 9.3	94.0 ± 3.0	67.3 ± 4.3
Chlorantraniliprole	32.3 ± 6.9	41.0 ± 14.0	97.7 ± 1.1	70.0 ± 2.1
Untreated control	26.7 ± 8.1	56.7 ± 9.8	95.7 ± 1.1	83.3 ± 6.8

<sup>a</sup> For *A. ipsilon*,  $F_{4, 20} = 1.32, 1.41, 1.52$  and  $P = 0.30, 0.26, 0.23$  for separate cohorts of 40 eggs at 1, 3 and 6 WAT respectively. For *P. japonica*,  $F_{4, 16} = 1.91$  and  $P = 0.16$ .



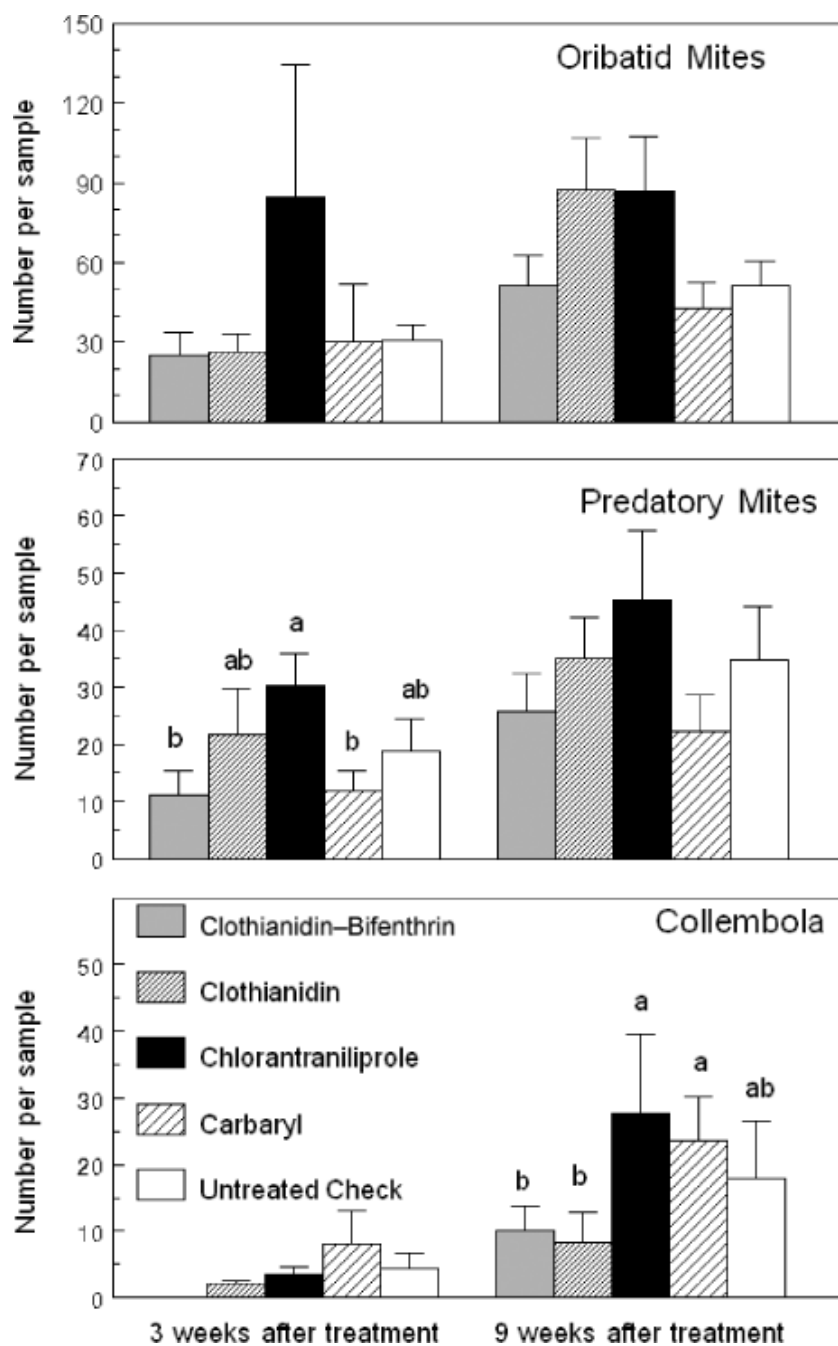


Figure 2.5. Abundance of soil microarthropods in samples from a golf course treated with representatives of different insecticide classes on 14 May 2010. Data are means ( $\pm$  SE) per plot. Post-ANOVA results are shown above those sets of bars for which the within-date treatment effect was significant ( $P \leq 0.05$ ). Within these sample intervals, bars not marked with the same letter differ significantly (LSD,  $P < 0.05$ ).

Table 2.3. Decomposition of tall fescue grass clippings in buried litter bags after plots on a golf course rough were treated with insecticides representing different chemical classes in May 2010

<b>Treatment</b>	<b>Grass clippings (mg dry weight) remaining after<sup>a</sup></b>	
	<b>2 months</b>	<b>4 months</b>
Clothianidin–bifenthrin	517 ± 71 a	207 ± 34 a
Clothianidin	620 ± 119 a	291 ± 75 a
Bifenthrin	159 ± 71 b	81 ± 46 ab
Chlorantraniliprole	47 ± 42 b	114 ± 55 ab
Untreated check	155 ± 69 b	30 ± 16b

<sup>a</sup> Data are means ± SE.  $F_{4, 20} = 8.3, 3.7$  and  $P < 0.01, 0.05$  for amounts remaining after 2 and 4 months respectively. Within columns, means not followed by the same letter are significantly different (LSD,  $P < 0.05$ ).

Table 2.4. Relative effectiveness of chlorantraniliprole versus bifenthrin application in reducing mounding activity by the ant *Lasius neoniger* on creeping bentgrass golf course tees

Treatment	Mounds (mean $\pm$ SE) per 6 m <sup>2</sup>		
	Pretreatment	1 WAT	3 WAT
<i>2010 trial</i>			
Chlorantraniliprole	26.0 $\pm$ 2.8	26.7 $\pm$ 4.2 a	11.2 $\pm$ 2.4 b
Bifenthrin	27.8 $\pm$ 2.8	3.2 $\pm$ 1.3 b	7.3 $\pm$ 1.7 b
Untreated check	27.3 $\pm$ 3.5	33.3 $\pm$ 4.9 a	20.8 $\pm$ 3.5 a
<i>2011 trial</i>			
Chlorantraniliprole	21.0 $\pm$ 3.4	14.0 $\pm$ 1.8 a	36.0 $\pm$ 8.7 a
Bifenthrin	22.6 $\pm$ 4.5	0.8 $\pm$ 0.4 b	8.2 $\pm$ 3.3 b
Untreated check	21.6 $\pm$ 4.4	23.8 $\pm$ 6.6 a	47.4 $\pm$ 11.5 a

For 2010,  $F_{2,8} = 0.6, 24.1, 9.7$  and  $P = 0.59, < 0.01, < 0.01$ ; for 2011,  $F_{2,8} = 0.5, 9.5, 13.2$  and  $P = 0.61, < 0.01, < 0.01$  for pretreatment and 1 and 3 weeks after treatment (WAT) respectively. Within years and columns, means not followed by the same letter are significantly different (LSD,  $P < 0.05$ ).

hypothesis that compared with bifenthrin, chlorantraniliprole provides little or no suppression of *L. neoniger* mounding (Table 2.4).

Chlorantraniliprole also did not reduce numbers of earthworms or casts compared with the untreated checks (Table 2.5). Carbaryl, as expected, significantly suppressed earthworm casting at 3 weeks after treatment, but the clothianidin–bifenthrin combination had even greater impact, reducing casts by 71 and 81% after 3 and 5 weeks respectively, and earthworm abundance by 78% for at least 5 weeks (Table 2.5). There was also a trend ( $P = 0.07$ ) on both sample dates for cast reduction following treatment with clothianidin alone.

## **Discussion**

Insecticide selectivity can be physiological, reflecting differences in uptake, detoxification or excretion, or ecological, arising from differential exposure (Croft 1990, Jepson et al. 1995). Assessment of pesticide hazards to beneficial species should therefore include trials realistically simulating how non-target organisms would be exposed in the field (Stark et al. 1995). The present trials, done in golf course settings with rates, timings and application methods similar to those used by turf managers, support the hypothesis that chlorantraniliprole causes less acute toxicity to epigeal predators, decomposers and some of their ecosystem services than are representatives of some other current classes of turf insecticides. The mode of entry of anthranilic diamides, including chlorantraniliprole, is mainly through ingestion; low contact activity contributes to their selectivity (Lahm et al. 2007). Chlorantraniliprole has shown low

Table 2.5. Mean ( $\pm$  SE) earthworm casts per m<sup>2</sup> at indicated weeks after treating plots on a soil-based putting green, and relative earthworm (*Apporectodea* spp.) abundance at the end of the 5 week trial

Treatment	Casts pretreatment	Number of casts after <sup>a</sup>		Worms per sample <sup>a</sup>
		3 weeks	5 weeks	
Carbaryl	35.3 $\pm$ 5.8	13.0 $\pm$ 2.5 c	39.2 $\pm$ 8.0 ab	6.8 $\pm$ 2.0 a
Clothianidin– bifenthrin	36.2 $\pm$ 7.0	9.7 $\pm$ 1.7 c	11.3 $\pm$ 2.9 c	2.5 $\pm$ 0.7 b
Clothianidin	38.2 $\pm$ 7.9	19.8 $\pm$ 2.4 b	26.3 $\pm$ 6.5 b	8.0 $\pm$ 2.1 a
Chlorantriniliprole	32.7 $\pm$ 5.9	21.2 $\pm$ 2.1 ab	42.5 $\pm$ 3.7 ab	13.0 $\pm$ 2.9 a
Untreated check	37.7 $\pm$ 6.1	33.5 $\pm$ 5.5 a	59.0 $\pm$ 15.8 a	11.2 $\pm$ 4.7 a

<sup>a</sup>  $F_{4,20} = 1.7, 9.7, 6.1, 3.9$  and  $P = 0.2, < 0.001, < 0.01, < 0.02$  for pretreatment, 3 and 5 week casts and worms per sample respectively. Within columns, means not followed by the same letter are significantly different (LSD,  $P < 0.05$ ).

toxicity to parasitoids (Brugger et al. 2009), entomopathogenic nematodes (Koppenhofer and Fuzy 2008), and pollinators (Dinter et al. 2009, Gradish et al. 2010) in laboratory studies. This study is the first to assess its effects on non-target invertebrates in turf.

The predominant families of epigeal predators in the present plots, which included ants, rove and ground beetles, Linyphiidae (sheet web and dwarf spiders) and Lycosidae (wolf spiders), are typical of those found in cool-season turf settings (Arnold and Potter 1987, Cockfield and Potter 1983, 1984, Terry et al. 1993, Smitley et al. 1998, Lopez and Potter 2003, Zenger and Gibb 2001). Chlorantraniliprole had little or no impact on any of these families, as determined by pitfall catches, which are composite measures of activity and abundance (Topping and Sunderland 1992) nor on predatory mites in the soil and thatch. In contrast, clothianidin, a representative neonicotinoid, and the neonicotinoid–pyrethroid premix had short-term impact on a number of predator groups. The temporary increase in pitfall trap captures of ants following application of the latter two products in late summer 2009 was likely due to sublethal neural excitation (Haynes 1988) and activity of foraging workers from exposure to the pyrethroid rather than increases in their populations. That view is consistent with the concurrent reduced predation on black cutworm eggs in plots treated with the neonicotinoid–pyrethroid combination. Workers of *L. neoniger* and other common turfgrass ants would have been at peak abundance in late summer when treatments for that trial were applied (Cockfield and Potter 1984, Maier and Potter 2005b).

Chlorantraniliprole also had no measured impact on populations of earthworms, Collembola or oribatid mites. These groups tend to be the predominant decomposers in cool-season turfgrass soils (Kunkel et al. 1999, Potter et al. 1990, Peck 2008, 2009). In

contrast, both clothianidin and the clothianidin–bifenthrin combination significantly reduced populations of both earthworms and Collembola in 2009, and the latter also affected earthworms in 2010. Both clothianidin and the combination product also significantly retarded grass clipping decomposition.

Turf managers seeking to control surface and subsurface pests with the same application can apply a combination formulation with two active ingredients or use a single chemistry such as chlorantraniliprole that is active against both lepidopteran and coleopteran pests. Multiple targeting is attractive to golf superintendents, lawn care providers and grounds managers because it simplifies treatment decisions and may also save time, labor and fuel costs (Houseworth 2009). Another purported benefit of combination products is potential synergistic or additive effects between the different chemistries (Kostromytska et al. 2011). Such effects, however, would also seemingly occur against beneficial invertebrates, and, indeed, more significant effects were seen on non-target arthropods and earthworms from the combination product than from either of its components alone. The present results indicate that chlorantraniliprole can provide the benefits of controlling multiple key pests without disrupting beneficial invertebrates and their activities.

Most insecticide-related reductions in activity or abundance of non-target invertebrates seen in this study lasted no more than a few weeks. Relatively rapid recovery of predatory invertebrates following insecticide perturbations is consistent with the pattern seen in earlier studies with older chemistries (Cockfield and Potter 1983, Terry et al. 1993, Kunkel et al. 1999, Peck 2008, 2009). Plot size in studies of this type is invariably constrained by trade-offs between need for replication, practical issues such as

amount of turf available and starting with reasonable consistency in site characteristics. Invertebrate repopulation of treated turf occurs both by immigration from surrounding areas and by reproduction of survivors or individuals originating from life stages not killed by the treatment. Plot sizes in the present main trials were large relative to the probable dispersal capabilities of earthworms and other decomposers, as well as smaller epigeal predators, but probably not for more mobile groups such as ground beetles that could recolonize treated areas when contact activity of residues declined. Although turf is a relatively resilient system, beneficial invertebrates would doubtless be slower to repopulate treated golf fairways, sports fields or home lawns than occurred in the present plots. Furthermore, repeated applications, within a growing season or over successive years, may have cumulative impacts on beneficial invertebrates (Peck 2008).

Ants and earthworms, beneficial in most turf settings, can become pests on golf courses and sports fields when their mounds or casts disrupt the smoothness and uniformity of playing surfaces, dull mower blades or smother small patches of grass. The present trials with clothianidin and previous ones with imidacloprid (Kunkel et al. 1999, Capowiez and Berard 2006) indicate that application of neonicotinoids for control of soil insects tends partially to suppress earthworms and casts. The clothianidin–bifenthrin premix had even greater impact on earthworms, greater than the impact of carbaryl. The trials on tees showed that applying a pyrethroid alone, done by many golf superintendents every 3–4 weeks during the growing season for cutworm control, secondarily suppresses ant mounds, albeit for only a few weeks after application. Because chlorantraniliprole does not suppress ants or earthworms, turf managers switching to this anthranilic diamide for its effectiveness against primary pests and favorable toxicology may need to spot treat



more often for those secondary problems that had been suppressed by formerly used less selective insecticides.

Worldwide industry initiatives towards more sustainable turf management will require effective pesticides that are both non-toxic to vertebrates and compatible with non-target invertebrates that contribute to pest suppression, decomposition of plant matter, nutrient recycling and good soil tilth (Potter 1993, Terman 1997, Racke 2000, Gange et al. 2003, Dobson 2005, Potter 2005, Held and Potter 2012, Bixby-Brosi and Potter 2012). This study indicates that, when compared with representatives of other classes of chemical insecticides, chlorantraniliprole has particularly low impact on terrestrial beneficial invertebrates and their services.

## **CHAPTER THREE**

### **Impacts of a neonicotinoid, neonicotinoid–pyrethroid premix, and anthranilic diamide insecticide on four species of turf inhabiting beneficial insects**

#### **Introduction**

Insecticides are used on lawns, golf courses, and sports fields to manage pests that diminish aesthetics and suitability for play (Blaine et al. 2012, Held and Potter 2012). Many turf managers prefer to preventively control foliage- and root-feeding pests with the same application to save fuel, time, and labor (Houseworth 2009, Held and Potter 2012, Buss 2012). That strategy, called multiple-targeting, may entail applying a tank mix or premix formulation with two or more active ingredients or a product with one active ingredient with broad activity (Buss 2012). Premixes used on turf typically contain a pyrethroid that binds to foliage and thatch and controls surface-active pests such as caterpillars and chinch bugs, and a neonicotinoid that, when watered-in, controls root-feeding pests such as scarab grubs and weevil larvae. Another purported benefit of premixes is the potential for target-site-based synergy (Elbert et al. 2008, Houseworth 2009, Kostromytska et al. 2011, Buss 2012). Synergism, if it occurs, might also cause premixes to be more toxic than their individual components to beneficial invertebrates (Gill et al. 2012). A clothianidin + bifenthrin premix, for example, suppressed earthworm populations and predation on sentinel black cutworm eggs more than did either of its components applied alone (Larson et al. 2012).

Chlorantraniliprole, the first anthranilic diamide labeled for turf usage (Held and Potter 2012), stimulates ryanodine receptors to release calcium stores from the

sarcoplasmic reticulum of insect muscle cells causing lethal paralysis in sensitive species (Cordova et al. 2006, Lahm et al. 2007). It controls all species of turf-damaging scarab grubs, as well as billbug and other weevil larvae, grass-feeding caterpillars and invasive crane flies (Held and Potter 2012). Its mode of entry is primarily by ingestion and secondarily by contact (Lahm et al. 2007; Brugger et al. 2010). It has high selectivity for insect ryanodine receptors (RyRs) when compared to mammalian RyRs (Cordova et al. 2006; Wang et al. 2012). Differences in target site (RyRs) sensitivity between, and in some cases within, insect orders may account for its activity against some insects but not others (Wang et al. 2012).

Turf supports many species of beneficial invertebrates including natural enemies (Cockfield and Potter 1984, Bixby-Brosi and Potter 2012a), decomposers (Potter et al. 1990, Potter 1993, Peck 2009), and pollinators that forage on flowering lawn weeds (Shepherd and Tepedino 2000, Larson et al. 2013). Turf industry initiatives toward sustainability (Racke 2000; Gange et al. 2003; Dobson 2005) are facilitated by conserving the services provided by beneficial invertebrates (Potter 1993, Held and Potter 2012, Bixby-Brosi and Potter 2012a, Larson et al. 2012). Previous work on ecotoxicology of turf insecticides has mostly used pitfall traps to assess impacts on predator activity-density, soil sampling for decomposers, or sentinel prey or mesh bags with plant litter to assess extent of disruption of predation or decomposition (e.g., Cockfield and Potter 1983, Terry et al. 1993, Kunkel et al. 1999, Zenger and Gibb 2001, Peck 2009, Larson et al. 2012). Only a few field studies have evaluated hazards of turfgrass insecticides to bees (Gels et al. 2002, Larson et al. 2013) or parasitoids (e.g., Rogers and Potter 2003, Oliver et al. 2006) none of which included pesticide mixtures.

We compared the impacts of clothianidin, a clothianidin + bifenthrin premix, and chlorantraniliprole on *Harpalus pennsylvanicus*, an omnivorous ground beetle, *Tiphia vernalis*, an ectoparasitoid of scarab grubs, *Copidosoma bakeri*, a polyembryonic endoparasitoid of black cutworms, and *Bombus impatiens*, a native bumble bee, using assays that simulated exposure to turf treated at label rates. Our a priori hypotheses were that the premix, because of its two active ingredients, would be more toxic than the neonicotinoid alone, and that chlorantraniliprole, because of its relative selectivity, would have lesser impact than the other chemistries on each of the indicator species.

## **Materials and Methods**

### **Insecticides**

Treatments included chlorantraniliprole (18.4% active ingredient; Acelepryn, Dupont, Wilmington, DE), clothianidin (50% active ingredient; Arena 50 WDG, Valent, Walnut Creek, CA), and a premix containing 24.7% clothianidin and 12.3% bifenthrin (Aloft, Arysta, Cary, NC). Within each assay the insecticides were applied at their label rates for preventive grub control (0.45 kg AI ha<sup>-1</sup> for clothianidin alone and in the premix; 0.23 kg AI ha<sup>-1</sup> for chlorantraniliprole). Lower rates were also included in the assay with *C. bakeri*, as explained below.

### **Acute Intoxication of a Ground Beetle by Insecticide-Treated Food**

*Harpalus pennsylvanicus* DeGeer (Coleoptera: Carabidae), a common ground beetle in turf settings (Kunkel et al. 2001) feeds on living or dead insects as well as seeds (Kirk 1973, Best and Beegle 1977). This assay simulated the beetles' scavenging on insecticide-contaminated seeds or dead insects such as might occur at treated sites.

About 200 wild adults were live-collected with black light traps in Fayette County, KY during July 2010. Beetles were kept in a glass aquarium with autoclaved, sifted soil, pieces of paper towels for shelter, and dog food pellets (Science Diet, Purina Mills, Graystone MO) as food (Kunkel et al. 2001). The aquarium was kept at ambient room temperature (21–23 °C) and 10:14 (L:D) photoperiod. Individual beetles were placed in plastic 118-ml diet cups containing sifted, autoclaved soil (6.35 mm deep) and starved for 36 h before the trial.

The insecticides were mixed with water at concentrations that would deliver their respective label rates (see above) if applied at 1628 liters per ha (4 gallons per 1000 ft<sup>2</sup>), a typical field spray volume. In this assay, bifenthrin alone (Talstar Select, 7.9% bifenthrin; FMC, Philadelphia, PA), a pyrethroid used to control surface-active turf pests, was also included to compare its activity with that of the premix. It was mixed at the concentration that would deliver the label rate for grass-feeding caterpillars (0.064 kg AI ha<sup>-1</sup>) if applied as above. A pipette was used to apply 10 µl of insecticide solution, or water alone, to individual dog food pellets (Science Diet; disc-shaped, 1.5 × 0.5 cm, about 1 g each), which then were allowed to dry overnight. This was intended to simulate beetles scavenging on food wetted by a field application of the tank mix. One pellet and an active, apparently healthy beetle were then added to each of 30 individual 237-ml plastic cups. There were 150 total beetles, 30 per treatment [clothianidin, premix, bifenthrin, chlorantraniliprole, and non-treated]. The cups were arranged in a randomized complete block design and kept at the temperature and photoperiod as the aforementioned stock colony. The beetles were checked at 1, 12, 24, 36 and 48 h and rated as normal, mildly intoxicated, or moribund/dead. Normal beetles showed no outward symptoms and

were able to right themselves from their back in <1 min; mildly intoxicated ones showed hyperactivity or paralysis of some legs, and took > 1 min to right themselves, whereas moribund/dead beetles were unable to right themselves and exhibited only slight twitching or no response to touch. The Gehan-Wilcoxon survival test, a nonparametric procedure for comparing two survival distributions (Lee 1992), was used to compare the mortality response over the 48-h trial from the premix with that from each of its components alone. To test for rapid additive or synergistic effects, the proportion of beetles intoxicated within 1 h by the premix, or by clothianidin or bifenthrin alone, were compared by Fisher's Exact test. All analyses were done with Statistix 8.0 (Analytical Software 2009).

### **Residual Effects on Parasitism of Root-Feeding Grubs**

The Japanese beetle, *Popillia japonica* Newman, is the most widespread and destructive pest of turf in the eastern United States causing more than \$450 million in damage each year (Vittum et al. 1999, Potter and Held 2000). *Tiphia vernalis* Rohwer (Hymenoptera: Tiphidae), an introduced parasitoid of Japanese beetle grubs, is native to Japan, Korea, and China (Fleming 1968). Adult *T. vernalis* are active from early May to early June in Kentucky (Rogers and Potter 2003) when they oviposit on overwintered third-instar hosts. Parasitism rates may be as high as 60% on central Kentucky golf courses, although 10–25% parasitism is more typical (Rogers and Potter 2003).

About 400 wild adult *T. vernalis* of mixed sex were collected from Pendleton Country Club in Pendleton Co., KY and University of Kentucky (UK) Spindletop Research Farm in Fayette Co., KY in May 2011 by spraying a 10% sugar water solution

on foliage of trees bordering turf areas (Rogers and Potter 2003) and capturing them with a hand-held vacuum (BioQuip, Gardena, CA). The wasps were kept in a clear canister containing 6.35 mm of sifted, autoclaved soil for 3 days before the trial. A dental wick soaked in 10% sugar water provided a food source. Young adult wasps can be kept alive for several weeks under those conditions (Rogers and Potter 2003).

Final instar *P. japonica* grubs were collected from a sod farm near Madison, WI in May 2011, shipped overnight to us, and left in the soil they had been collected into for 2 d before use. Forty-eight cores of Kentucky bluegrass, *Poa pratensis* L. with soil (10.2 cm diameter, 12 cm deep) were extracted with a golf course cup cutter from an untreated turf stand and put into plastic pots. Disposable hand sprayers were used to apply clothianidin, the clothianidin-bifenthrin premix, or chlorantraniliprole to the grass at the aforementioned label rates and spray volume. There were 12 replicates (cores) for each insecticide, plus untreated checks. Five grubs were introduced to each core 24 h after treatment by inserting them into slits cut into the side just below the thatch so that they would not directly contact insecticide residues on the turf surface. Clear 1-liter plastic cups with screened bottoms were inverted over each pot to form a ventilated cage.

Two active female *T. vernalis* wasps collected < 48 h earlier, and a dental wick with 10% sugar water, were added to each cage 24 h after the grubs were introduced. The caged cores were held in a growth chamber at 26 °C and 14:10 h (L: D) photoperiod for 7 d, after which the turf and soil were examined and still-living wasps and grubs with or without *Tiphia* eggs or larvae attached to their ventral surface were counted. Data were subjected to analysis of variance (ANOVA) for a completely randomized design, or in the case of variables that had all zeroes (no variance) for some treatments, the non-parametric

Kruskal-Wallis test. Log or square-root transformations were used as needed to correct for heterogeneity of variance; the arcsine square-root transformation was used with percentages. Data are presented as original means  $\pm$  standard error (SE).

### **Contact Toxicity to an Encyrtid Parasitoid of Black Cutworm**

The black cutworm, *Agrotis ipsilon* Hufnagel, is a major pest of golf course putting greens, tees, and other low-cut sports turf (Williamson and Potter 1997). The larvae chew down the grass surrounding their burrows, reducing aesthetics and uniformity of playing surfaces. *Copidosoma bakeri* Howard (Hymenoptera: Encyrtidae) is a small (about 1 mm long) polyembryonic wasp that oviposits into eggs of many species of noctuid moths (Schaaf 1972, Byers et al. 1993), including black cutworm, in turf (Bixby-Brosi and Potter 2012b). Once the host larva has eclosed, the wasp egg divides multiple times giving rise to all-male or all-female broods consisting of hundreds or thousands of clonally identical larvae that consume and kill the host from within during its final larval stadium (Saeki et al. 2009, Bixby-Brosi and Potter 2012b). Adult *C. bakeri* emerge from host cadavers in the thatch, mate, and search for host eggs on turfgrass leaf blades. Because of the wasps' small size, dispersal likely is limited, so they may be unable to avoid exposure to dry insecticide residues on treated turf. We used a non-choice contact assay that simulated such exposure but allowed accurate counting of living and dead wasps which would be difficult or impossible in living, dense turf.

Wasps were obtained from a laboratory colony maintained by published methods (Saeki et al. 2009, Bixby-Brosi and Potter 2012b). Filter paper (Whatman #1) cut into squares (8  $\times$  8 cm) was sprayed with the insecticidal solutions and left overnight to dry.



The papers were then rolled and placed in clear glass vials (29.6 ml; 95 × 25 mm). Then cohorts of about 30–50 wasps were aspirated into the vials. A cotton plug prevented their escape. There were six replicates per rate. Because in a preliminary trial, exposure to one-half label rates of clothianidin or the premix killed 100% of the wasps within 1–3 h, lower rates were included in the main experiment to test if, with exposure to lesser amounts such as might occur some weeks after residues had begun to dissipate in the field, the premix would cause higher mortality than would clothianidin alone. Eight treatments were included in the main experiment: chlorantraniliprole at full label rate, clothianidin alone and in the premix at 0.5, 0.25, and 0.125× label rates, and the untreated check. Knockdown was visually assessed after 1 h, and percentage mortality was quantified after 3 h by microscopically examining the wasps. Data were analyzed by ANOVA for a completely randomized design followed by pre-planned orthogonal contrasts (1 df) to compare mortality from chlorantraniliprole versus the untreated check, chlorantraniliprole versus clothianidin (all rates), clothianidin versus the premix (all rates), and clothianidin versus the premix at the lowest tested (1/8th label) rate.

### **Acute Effects on Bumble Bee Colonies Exposed to Residues on Flowering Weeds**

Label precautions state not to apply clothianidin, the premix, or chlorantraniliprole to flowering plants where bees may forage. However, when those products are applied in spring to preventively control grubs and other pests, flowering lawn weeds such as dandelions (*Taraxacum officinale* Higg) and white clover may be oversprayed along with the turfgrass (Larson et al. 2013). We simulated that scenario to compare the insecticides' acute impacts on colonies of a common native bumble bee, *Bombus impatiens* Cresson exposed in that manner. The main results of this trial were

previously reported (Larson et al. 2013) as part of a larger study that compared both short-term and reproductive effects of exposure to clothianidin or chlorantraniliprole residues on *B. impatiens*. Although not the focus of that study, the clothianidin-pyrethroid premix was included in the short-term trial. Because it is relevant to the present study, we contrast here the immediate impacts of clothianidin and the premix on selected measures of colony vigor, a comparison not previously reported.

The study site was a sward of Kentucky bluegrass with about 30% cover (by visual estimate) of flowering white clover at the University of Kentucky's A.J. Powell, Jr. Turfgrass Research Facility, near Lexington. Plots ( $3.35 \times 3.35$  m) were situated on areas with similar clover density and were at least 2 m apart. Treatments were chlorantraniliprole, clothianidin, or the premix at their aforementioned label rates. The applications were made by a professional lawn care provider ( $410 \text{ L ha}^{-2}$  spray volume) under the authors' supervision on 1 June 2011. Residues either were allowed to dry on the turf and flowering clover, or were watered in (30.3 liters per plot from sprinkling cans). There were five replicates of each insecticide for both non-irrigated and irrigated plots, plus untreated controls. Open-bottom screen enclosures ( $3.05 \times 3.05$  m, 2 m tall; Instant Screen Shelters; Coleman, Wichita, KS) were erected on each plot the following day.

Commercial bumble bee colonies (Research Mini-hives; Koppert; Howell, MI), each initially containing 20 workers and a fertilized queen housed in a plastic hive within an outer cardboard box, were blocked by their initial weights and randomly assigned to the treatments. One colony was placed centrally inside each enclosure. The bees were introduced on the evening of 2 June 2011 and left to forage on clover blooms in the

enclosed turf plots until 15 June when, after dark and foraging workers had returned, the hive doors were closed. Colonies were then taken to the lab, stored at 4°C, and dissected by replicate over the next 10 days. Because the main effect of irrigation was non-significant ( $P > 0.2$ ) for all dependent variables, data from irrigated and non-irrigated plots were combined for ANOVA comparing numbers of living and dead workers and brood, and number of honey pots (waxy honey receptacles constructed by bees). Mean separation was by Fisher's least significant difference (LSD) test ( $P < 0.05$ ).

## **Results**

### **Acute Intoxication of a Ground Beetle by Insecticide-Treated Food**

Beetles were observed feeding on all treated food pellets. Most of the ones provisioned with food treated with clothianidin, bifenthrin, or the premix were intoxicated or dead within 24 h (Table 3.1). Gehan-Wilcoxon survival tests indicated a similar temporal pattern of mortality from the premix and clothianidin alone ( $Z = 0.70$ ,  $P = 0.49$ ), but faster mortality from the premix than from bifenthrin ( $Z = 2.52$ ,  $P < 0.01$ ). The premix intoxicated proportionately more beetles than either component after 1 h, and it killed proportionately more beetles than bifenthrin after 12 h (Fisher's exact test,  $P \leq 0.05$ ). Beetles provided chlorantraniliprole-treated food suffered no mortality or acute intoxication.

### **Residual Effects on Parasitism of Root-Feeding Grubs**

No grubs were parasitized by *T. vernalis* in turf cores with residues of the neonicotinoid-pyrethroid premix. Parasitism was also significantly reduced by clothianidin alone (Table 3.2). Similar numbers of living, apparently healthy *P. japonica*

**Table 3.1**

Number of ground beetle (*H. pennsylvanicus*) adults, out of 30, displaying progressive poisoning symptoms during 48 hour access to insecticide-contaminated food (see text for survival analysis).

Exposure duration (h)	Clothianidin			Bifenthrin			Premix <sup>a</sup>			Chlorantraniliprole			Untreated		
	L	I	D	L	I	D	L	I	D	L	I	D	L	I	D
1	29	0	1	30	0	0	23	7	0	30	0	0	30	0	0
12	5	12	13	14	7	9	7	3	20	30	0	0	30	0	0
24	2	9	19	7	9	14	1	8	21	29	0	1	30	0	0
36	1	10	19	4	6	20	1	8	21	29	0	1	30	0	0
48	1	10	19	4	5	21	1	7	22	29	0	1	30	0	0

L= live, I= intoxicated, D= dead

<sup>a</sup>Premix contains clothianidin + bifenthrin

**Table 3.2**

*Tiphia vernalis* parasitism of Japanese beetle grubs in the root zone of turf cores that had been treated with a neonicotinoid (clothianidin), anthranilic diamide (chlorantraniliprole) or premix (clothianidin + bifenthrin) insecticide 48 h before introducing the wasps

Treatment	Live grubs recovered <sup>a</sup>	Parasitized grubs <sup>b</sup>		Total parasitized grubs
		No. per core	Percentage	
Clothianidin	4.8 ± 0.2	0.4 ± 0.2*	8.8 ± 3.2*	5
Premix	4.8 ± 0.4	0*	0*	0
Chlorantraniliprole	4.2 ± 0.4	2.0 ± 0.3	41.0 ± 6.4	24
Untreated	4.9 ± 0.2	2.1 ± 0.5	40.6 ± 8.4	25

Data are means (± SE)

<sup>a</sup> Five grubs initially were introduced to each core; number of live grubs recovered did not differ among treatments ( $F_{3,44} = 1.2$ ;  $P = 0.33$ )

<sup>b</sup> Number and percentage of parasitized grubs differ among treatments (Kruskal-Wallis test,  $P < 0.001$ ). Marked means (\*) significantly lower than untreated check (Dunnett's test,  $\alpha = 0.05$ ).

grubs were recovered in all treatments suggesting that the surface-applied residues of clothianidin and the premix had intoxicated the wasps as opposed to rendering the white grubs unsuitable as hosts. Chlorantraniliprole did not reduce parasitism compared to the untreated control (Table 3.2). Only a few adult wasps were still alive by the time the cores were dissected to examine the grubs for *T. vernalis* eggs or larvae.

### **Contact Toxicity to an Encyrtid Parasitoid of Black Cutworm**

Adult *C. bakeri* were quickly intoxicated by exposure to dry residues of clothianidin or the premix, even at 1/8th of the label rates ( $F = 78.7, 62.4$  at 1 and 3 h, respectively;  $df = 7, 32$ ;  $P < 0.001$ ; Figure 3.1). Although exposure to residues of clothianidin or of the premix caused similarly high mortality after 3 h ( $t = 1.1$ ;  $P = 0.29$ ), there was somewhat faster (1 h) knockdown from the premix across all rates ( $t = 3.28$ ,  $P < 0.005$ ) and at the lowest (1/8 label) rate tested ( $t = 3.35$ ,  $P < 0.005$ ; Figure 3.1). Chlorantraniliprole caused no knockdown or mortality; survival of those cohorts was similar to the untreated check (linear contrasts;  $t = 0.92, 0.98$ ;  $P > 0.33$ ).

### **Acute Effects on Bumble Bee Colonies Exposed to Residues on Flowering Weeds**

Colonies that foraged on flowering clover in turf treated with clothianidin or the premix had similar numbers of live workers, dead workers, live and dead immature life stages (combined larvae and pupae), and honey pots (Figure 3.2). Both treatments significantly reduced the numbers of live workers, and resulted in more dead ones, compared to the untreated controls. There also was a trend for colonies exposed to either treatment to have fewer total live bees (all life stages besides eggs;  $F_{2, 22} = 2.85$ ,  $P = 0.08$ ). Colony weights after the 2 weeks of foraging on flowering clover in clothianidin-,

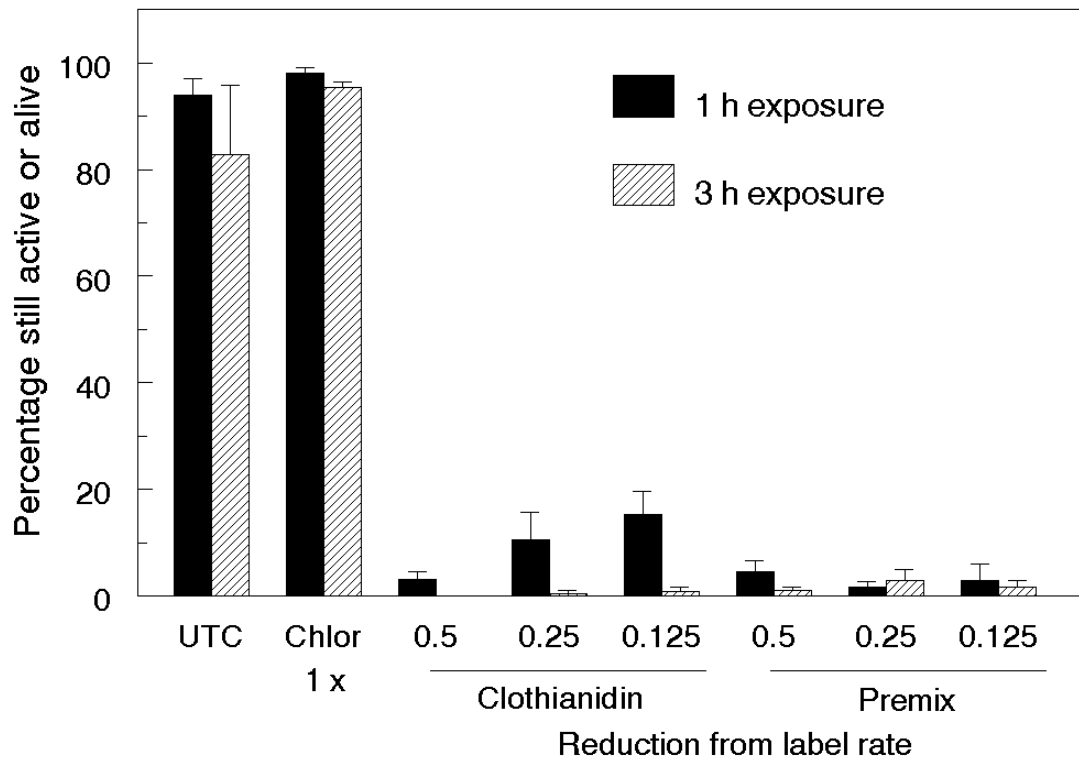


Figure 3.1. Response of cohorts of 30–50 adult *Copidosoma bakeri* exposed to dry residues of turfgrass insecticides showing knockdown (1 h) and mortality (3 h) from reduced rates of clothianidin or a clothianidin - bifenthrin premix compared to the untreated control (UTC), and absence of intoxication from chlorantraniliprole (Chlor). See text for statistical analysis.

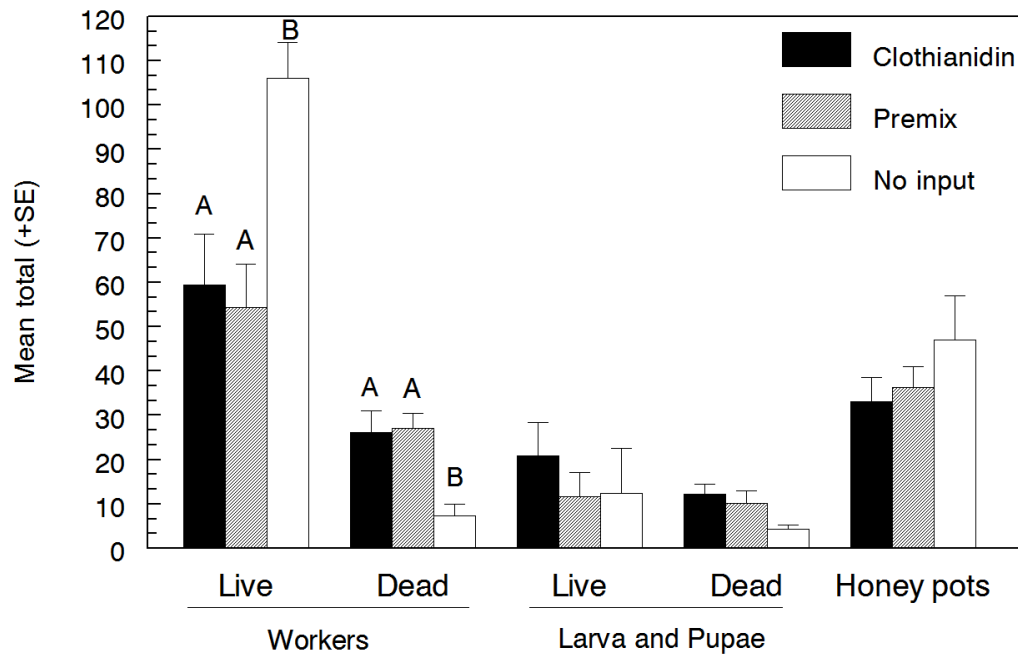


Figure 3.2. Condition of *Bombus impatiens* colonies sacrificed and examined after 2 weeks' foraging on flowering white clover blooms that had been sprayed with clothianidin or a clothianidin  $\pm$  bifenthrin premix when the weedy turf was treated at label rates for grub control. Both insecticides caused similar extent of acute reduction in number of live workers ( $F_{2,22} = 4.90$ ,  $P = 0.02$ ) and increase in dead workers ( $F_{2,22} = 4.96$ ,  $P = 0.02$ ) compared to the untreated controls. Within those groups, means not accompanied by the same letter significantly differ (LSD,  $P < 0.05$ ). Numbers of live immature bees ( $F_{2,22} = 0.48$ ,  $P = 0.62$ , dead immatures ( $F_{2,22} = 2.34$ ,  $P = 0.12$ ), or wax honey pots ( $F_{2,22} = 1.11$ ,  $P = 0.35$ ) were not affected within the time frame of the experiment.



premix-, or non-treated turf were  $580 \pm 17$ ,  $554 \pm 14$ , and  $602 \pm 6$  g, respectively, and did not significantly differ among treatments ( $F_{2,22} = 1.89$ ,  $P = 0.17$ ). Colonies exposed to chlorantraniliprole residues showed no acute adverse effects compared to the controls (data presented in Chapter Four).

## **Discussion**

Beneficial invertebrates in turfgrass may be exposed to insecticides by being directly sprayed, by contacting residues on foliage or in thatch or soil, by feeding on treated prey or seeds, or by imbibing nectar or guttation water (Potter 1993, Kunkel et al. 2001). Mobile natural enemies (e.g., ground beetles, parasitic wasps) or pollinators could encounter residues when venturing from non-treated lawns or golf course roughs into treated lawns or fairways. Our trials indicate that clothianidin and a clothianidin-bifenthrin combination premix, representing two of the three insecticide classes used for preventive control and multiple targeting of turf insect pests, have the potential to intoxicate a range of beneficial insects that encounter their residues in turfgrass settings at label rates. Chlorantraniliprole residues, in contrast, had no apparent adverse effects on any of the beneficial species. Those results support those from an earlier field study (Larson et al. 2012) wherein the insecticides were applied to plots in golf course roughs and activity-density of surface-active predatory arthropods was assessed with pitfall traps. Acute impact of clothianidin and the premix, but not chlorantraniliprole, on bumble bees foraging in turf with flowering weeds that were sprayed supports previous findings (Larson et al. 2013).

Contrary to our hypothesis, the combination formulation, a clothianidin + bifenthrin premix, did not cause greater acute mortality of any of the beneficial insects in our assays more so than did clothianidin alone. The only suggestion of greater activity from the premix was its somewhat faster incapacitation of *H. pennsylvanicus* and knockdown of *C. bakeri* compared to clothianidin alone.

Hazards to parasitoids (e.g., *T. vernalis*) from insecticides used to preventively control multiple insect pests can sometimes be mitigated by adjusting application timing so as not to coincide with seasonal activity of the adults (e.g., Rogers and Potter 2003). Strict adherence to the insecticides' label precautions not to apply them to blooming, pollen-shedding, or nectar-producing parts of plants that may be visited by bees during the specified interval will reduce hazard to pollinators. In the case of lawn treatments, controlling spring-flowering weeds beforehand, mowing to remove flower heads before or immediately after the application, or waiting until after such weeds have finished blooming will reduce hazard to bees (Larson et al. 2013). Use of granular formulations, as opposed to sprays, may reduce exposure of bees and above-ground parasitoids to residues (Gels et al. 2002). Using target-selective insecticides is another way to reduce hazards to beneficial invertebrates in turf.

Absence of adverse effects from chlorantraniliprole in this study is consistent with lab studies indicating the low acute toxicity of field-relevant dosages of that compound to bumble bees (Dinter et al. 2009, Gradish et al. 2010) and parasitoids of field crop pests (Brugger et al. 2010, Liu et al. 2012). Chlorantraniliprole does have high acute oral toxicity to larval scarabs and weevils (Held and Potter 2012) and to Japanese beetle adults (Redmond and Potter, unpublished data), so our data showing absence of acute

oral effects on *H. pennsylvanicus* is noteworthy in suggesting selective activity within the order Coleoptera.

Preventive insecticides, including neonicotinoids, neonicotinoid-pyrethroid premixes, and chlorantraniliprole, have become mainstays for controlling many key turfgrass pests (Held and Potter 2012). Over-reliance on one chemical class can also promote insecticide resistance (Cherry and Nagata 2007, Ramoutar et al. 2009). Although the reductions in activity-density of beneficial turf invertebrates following application of neonicotinoids or premixes tend to be transitory (Kunkel et al. 1999, Larson et al. 2012), they may temporarily disrupt plant litter decomposition by soil invertebrates or biological control of pests (Potter et al. 1990, Potter 1993; Zenger and Gibb 2001, Rogers and Potter 2003, Larson et al. 2012), potentially contributing to pest resurgence or secondary pest outbreaks (e.g., López and Potter 2000). Moreover, their adverse effects, particularly on the soil fauna, may be compounded by consecutive years of use (Peck 2009).

Neonicotinoids and pyrethroids also are intrinsically toxic to bees (Blacquièrre et al. 2012). Preventive insecticides are typically applied to lawns in the spring when flowering weeds may be present and new bumblebee queens are foraging. Exposure to flowers contaminated by such sprays could potentially reduce localized bee populations. The public's increasing awareness of the potential hazards of pesticides to bees and other non-target species is fueling an industry-wide shift toward turf management practices that promote a healthy environment (Terman 1997, Carpenter and Meyer 1999, Kenna and Snow 2000). Target-selective insecticides that control pests without harming pollinators or disrupting biological control by endemic natural enemies are important to those initiatives (Held and Potter 2012, Larson et al. 2012).

Two commonly used preventive turf insecticides, a neonicotinoid-pyrethroid premix and a neonicotinoid alone, were acutely toxic to all of the beneficial insects used in our trials, whereas the anthranilic diamide did not adversely affect any of them. Compared to neonicotinoids, chlorantraniliprole has similar strong efficacy against scarab and billbug larvae, better activity against caterpillars, but is less active against sucking pests (Held and Potter 2012). It appears to be a good fit for preventively controlling multiple turf insect pests without disrupting beneficial invertebrates and their services.

## **CHAPTER FOUR**

### **Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns**

#### **Introduction**

Native bee and honey bee populations are declining due to habitat loss and fragmentation, disease, and other stresses (Potts et al. 2010, Goulson et al. 2008, Cameron et al. 2011, Hernandez et al. 2009, Hopwood et al. 2012). Bees in cities and suburbs survive by gathering nectar and pollen from flowering plants in lawns, gardens, and patches of semi-natural habitat (Goulson et al. 2008, Cameron et al. 2011, Hernandez et al. 2009, Hopwood et al. 2012, McFrederick and Lebuhn 2006). In the United States, where about one million hectares of farmland and natural habitat are converted to urban areas each year (McFrederick and Lebuhn 2006), turf grasses now cover about 164,000 km<sup>2</sup>, an area three times larger than any irrigated agricultural crop (Milesi et al. 2005). Most of this area (>75%) is comprised of residential, commercial, and institutional lawns. Some lawns are treated with insecticides by homeowners or commercial lawn care providers (Held and Potter 2012, Blaine et al. 2012).

Neonicotinoids, systemic insecticides that move via sap throughout treated plants, are potent selective agonists of nicotinic acetylcholine receptors in insects (Tomizawa and Casida 2003). Imidacloprid, clothianidin, and thiamethoxam are widely used on lawns (Held and Potter 2012). Typically applied as sprays or granules in spring and leached into the soil by irrigation or rainfall, they provide several months of residual control of root-feeding grubs and other pests (Held and Potter 2012). Despite label

precautions stating not to apply neonicotinoids to plants in bloom, applications are sometimes made when lawn weeds such as dandelions and white clover are flowering. These weeds are attractive to native pollinators, especially bumble bees, and to managed and feral honey bees (Morse et al. 1990, Gels et al. 2002, Tomasi et al. 2004).

Although residue levels in nectar and pollen of neonicotinoid-treated crops tend to be below acute toxicity levels for bees (Bonmatin et al. 2003, 2005, Blacqui re et al. 2012), lethal and sublethal effects of dietary exposure including impaired learning, memory, and navigational abilities of honey bees (Hopwood et al. 2012, Blacqui re et al. 2012, Decourtye et al. 2004, Desneux et al. 2007, Henry 2012) and reduced foraging, colony growth, and queen production by bumble bees (Mommaerts et al. 2010, Whitehorn et al. 2012, Gill et al. 2012, Laycock et al. 2012) have been described. Most of the evidence, however, comes from studies in which doses of the insecticide were lab-fed to bees in sugar water or pollen, and in some such trials, dosages typical of those found in seed-treated crops had no observed adverse effects (Tasei et al. 2000, Franklin et al. 2004). Some field studies in which bees were exposed to crops grown from neonicotinoid-treated seeds failed to detect detrimental effects on colony health (Tasei et al. 2001, Cutler and Scott-Dupree 2007). Bumble bee colonies exposed to dry spray residues of imidacloprid on weedy turf gained less weight and produced fewer workers, brood chambers, and honey pots compared to controls, but when spray residues were watered into the soil, or the insecticide was applied in granular form, no adverse effects on those measures of colony health were observed (Gels et al. 2002). The extent to which trace dietary neonicotinoids impact bees in field settings remains controversial and

requires studies with relevant exposure and duration to resolve (Cresswell 2011, Blacqui re 2012).

Anthranilic diamides are a relatively new class of insecticides that activate insect ryanodine receptors by stimulating release of calcium stores from muscle cells causing lethal paralysis in sensitive species (Cordova et al. 2006). They have low vertebrate toxicity, low use rates, and 3–5 month residual activity in soil, as well as low impact on non-target invertebrates (Cordova et al. 2006, Brugger et al. 2010, Larson et al. 2012). Chlorantraniliprole, the first anthranilic diamide lawn insecticide, received reduced-risk status from the US Environmental Protection Agency. Compared to neonicotinoids, it has similar efficacy against root-feeding scarab grubs and weevil larvae, better activity against caterpillar pests, but is less active against chinch bugs (Held and Potter 2012). Chlorantraniliprole has low acute adult bee toxicity (Cordova et al. 2006) but its potential reproductive effects on bees with realistic field exposure have not been evaluated. If benign, it could be a more bee-friendly option for insect control in lawns, gardens, and other settings where bees are active.

We exposed colonies of the bumble bee *Bombus impatiens* to turf intermixed with white clover where clothianidin or chlorantraniliprole had been applied at label rates to test the hypothesis that the latter is relatively less hazardous to colonies foraging on flowering weeds in treated lawns. Several scenarios were used to assess the insecticides' respective impacts on colony health and queen production. Our results showed that colonies foraging on the neonicotinoid-treated turf had higher worker and brood mortality, reduced honey pot production, delayed weight gain, and impaired queen production compared to controls, but also suggested that the hazard is reduced after

blooms present at the time of application are removed by mowing. The anthranilic diamide appears to be non-hazardous to bumble bees even when used on lawns where flowering weeds are present.

## **Materials and Methods**

### **Insecticide impacts on foraging, colony health and queen production**

This trial evaluated the scenario of resident bees foraging on flowering weeds in a newly-treated lawn for six days before the turf was mowed. The exposure phase was done at the A.J. Powell Turf Research Center, University of Kentucky, near Lexington, KY in a 1-ha sward of Kentucky bluegrass (*Poa pratensis* L.) with about 30% cover (by visual estimate) of flowering white clover (*Trifolium repens* L.). Plots ( $3.35 \times 3.35$  m; 10 replicates of each insecticide) were situated on areas with similar clover density and were at least 2 m apart. Treatments were clothianidin (Arena 50 WDG; Valent, Walnut Creek, CA), chlorantraniliprole (Acelepryn, 18.4% active ingredient (AI); Dupont, Wilmington, DE), and the untreated check. Both products were applied as they would be for scarab grub control at their high label rates, 0.45 and 0.23 kg AI ha<sup>-1</sup> for clothianidin and chlorantraniliprole, respectively. The applications were made on 14 May 2012. We used a portable CO<sub>2</sub> spray tank (R and D Sprayers, Opelousas, LA) equipped with a 1.8 m handheld boom with four Spraying System 8004 Tee Jet nozzles (Spraying Systems, Wheaton, IL) that delivered a pressure of 2109 g cm<sup>-2</sup>. Spray volume was 468 L ha<sup>-1</sup>, applied by making two passes in opposite directions over each plot. Separate spray bottles were used for each treatment. Residues were lightly watered in (30.3 liters per plot) from sprinkling cans about 1 h after application.



Screen enclosures ( $3.05 \times 3.05$  m; Instant Screen Shelters, Coleman; Wichita, KS) were erected on each plot 24 h after application. Commercial *Bombus impatiens* colonies (Research Mini-hives; Koppert, Howell, MI), one per enclosure (10 per treatment), were randomly assigned to the treatments after being blocked by their initial weight. Each colony was housed within a plastic hive within an outer cardboard box and started with 20 workers and a fertilized queen. Colonies were shipped with a syrup food sack which was left in the hives while they were confined on the weedy turf plots but removed when the bees were moved to the safe foraging site (see below). Colonies were introduced to the enclosures on 16 May two days after the insecticides had been applied. Each enclosure was inspected in mid-afternoon on the 5th and 6th day after introduction and workers foraging within the enclosed area at that time were counted. After six days the doors of foraging the hive doors were closed at night, after workers had returned. Nest materials cannot be removed from the inner plastic hive without causing severe disturbance so they were weighed together. Hives were weighed after closure (23 May), replaced in their boxes, and then transported 12 km to Gainesway Farm (Lexington, KY) a 700 ha working horse farm at which no insecticides are applied to the pastures, grounds, or trees. The colonies were placed on concrete blocks at least 3 m apart along the edge of a woodlot. Their openings faced a pasture with wildflowers including clover. The site was at least 1 km from the nearest edge of the farm. Gainesway Farm is surrounded by pastures of other horse farms where no pesticides are applied, making it highly unlikely that foraging workers would be exposed to additional insecticides.

Colonies were left to openly forage at the horse farm site for 6 more weeks. They were inspected and weighed in the field on 31 May and 13 June. They were closed on 3

July, brought to the lab, and held at 4.4 °C until evaluated. Colonies were weighed and then dissected, by replicate, over the following 1.5 weeks to assess numbers of living and dead adults (combined workers and males), queens, honey pots, and living and dead larvae and pupae, and weights of live adults and queens.

Samples of 100 presumably non-pollinated flowers (i.e., lacking drooped brown basal florets indicative of having been pollinated) were collected from each of five replicates of the clothianidin-treated plots after the bees were removed on the 6th day after treatment. Because nearly all blooms in chlorantraniliprole and control plots appeared to be pollinated, samples of 100 non-pollinated flowers were collected from each of five distinct untreated areas outside the enclosures but in the same turf sward. Florets were trimmed with scissors and then whole individual flowers were inverted and centrifuged in individual 15 ml centrifuge tubes for 10 min at 2000 rpm to extract the nectar. Nectar samples (about 300 mg per 100 flowers) were consolidated within each plot, transferred to micro-centrifuge tubes, and sent to the USDA-AMS National Science Laboratory (Gastonia, NC) where they were analyzed for clothianidin residues (1 ng/g level of detection) by high performance liquid chromatography separation coupled with a tandem mass selective detection system (LC-MS/MS) following a modified version of the AOAC official method of analysis 2007.06 (QuEChERS method) (Payá et al. 2007).

#### **Acute effects of exposure to insecticide residues before and after mowing**

This study was done on a different part of the same sward used for the exposure phase of the previously-described trial, using similar methods, except as follows. The treatments were made by a professional care applicator, supervised by the authors, on 1

June 2011. The insecticides were diluted in water and applied with a lawn spray gun (model 11-857-00 Mag 2000; 7.6 liters/min nozzle; GNC Industries, Pocahontas, AR) powered by an electric pump (FloJet model 4300-405; FloJet, Irvine, CA) at their label rates for grub control. Spray volume was 410 L ha<sup>-2</sup>. Residues were either allowed to dry on the surface, simulating what typically occurs with commercial lawn applications, or were watered in as described earlier. The open-bottom screened enclosures were erected on each plot 24 h later, and a commercial *B. impatiens* colony consisting of 20 workers and a fertilized queens as described above, was introduced to each enclosure that evening. Colonies were left to forage in the enclosures for two weeks before the hives were closed and brought to the lab for evaluation. The sward then was mowed (2.5 cm cutting height) to remove clover flowers present at the time of treatment. One week later (22 June), after new blooms had formed, another set of freshly-shipped bee colonies (Research Mini-hives; Koppert, Howell, MI) of the same size and age as those used for the initial challenge was introduced and left to forage in the enclosures for two weeks, after which the hives were closed and brought to the lab for evaluation. Initially there were five replicates for each combination of insecticide and watering regime, plus untreated controls, but because the irrigation main effect was non-significant for all dependent variables, data from irrigated and non-irrigated plots were combined for analysis.

## **Avoidance**

This study was done on a 2.5 ha sward of non-irrigated Kentucky bluegrass intermixed with white clover at the University of Kentucky's intramural sport field complex. Plots (3.7 × 3.7 m) were treated with clothianidin or chlorantraniliprole at label

rates on 23 May 2012, using the portable CO<sub>2</sub> sprayer described earlier. Those treatments, plus untreated plots, were arranged in a randomized complete block with five replicates per treatment (i.e. 15 plots in total). Untreated borders (2.44 m) surrounded each plot. Residues were not watered in, and there was no rainfall during the trial. Bee counts were taken daily for 1 week between 10:30 and 16:00 by slowly walking around each plot, staying in the border, and counting honey bees (*Apis mellifera*) and bumble bees (*Bombus* spp.) foraging on the clover. Each plot was observed for 1 minute, and after all plots had been inspected, the census was repeated, starting at the first plot, providing two counts within a 45-minute period. Bees moved from plot to plot, and between border areas and plots, so each count represented a snapshot of bees on a plot at that time.

### **Statistical Analyses**

Numbers of foraging workers in field enclosures, final colony weights, and parameters measured during dissections were compared among treatments by analysis of variance (ANOVA), followed by pre-planned orthogonal contrasts to compare each of the individual insecticides to the untreated control. We used the angular transformation for percentages and square root or log transformations for those data sets where treatment variances were non-homogeneous. Non-parametric tests were used for number of new queens where ANOVA assumptions were not met. Colony weights over time were compared using repeated measure ANOVAs. Counts of bees observed in the avoidance trial plots were totaled across census dates and analyzed by two-way ANOVA. All data are given as original means  $\pm$  SE. Statistix 9 was used for analyses.

## **Results**

Colonies exposed to clothianidin-treated weedy turf showed reduced foraging activity and increased worker mortality in the hives within five days (Figure 4.1). They also gained weight more slowly after being moved to an insecticide-free site where they were left to openly forage for six more weeks (Figure 4.2). Although statistically significant differences were no longer detected by analysis of variance by the time the hives were dissected, there remained consistent trends for fewer live adults (workers and males), honey pots, and reduced colony weight of clothianidin-exposed colonies compared to the controls ( $P = 0.052, 0.09, 0.058$ , respectively; pre-planned linear contrasts, Table 4.1). More importantly, clothianidin-exposed colonies failed to produce new queens (Figure 4.3). Chlorantraniliprole-exposed colonies showed no impairment in weight gain or reductions in other indicators of colony health, including new queen production, compared to the controls (Figure 4.3, Table 4.1),

Nectar extracted by centrifugation from 100-flower samples of clover flowers from the clothianidin-treated plots one week after application in 2012 contained  $171 \pm 44$  ng/g clothianidin (range 89–319;  $n = 5$ ), whereas nectar samples from flowers in open, non-treated areas contained no detectable insecticides. Nearly all of the flowers under the enclosures on non-treated or chlorantraniliprole-treated plots had been pollinated which precluded collecting sufficient nectar from them for analysis.

In another set of trials, *B. impatiens* colonies evaluated after two weeks' exposure to clothianidin-treated turf with flowering white clover suffered significantly higher worker and brood mortality and produced fewer honey pots, whereas colonies similarly

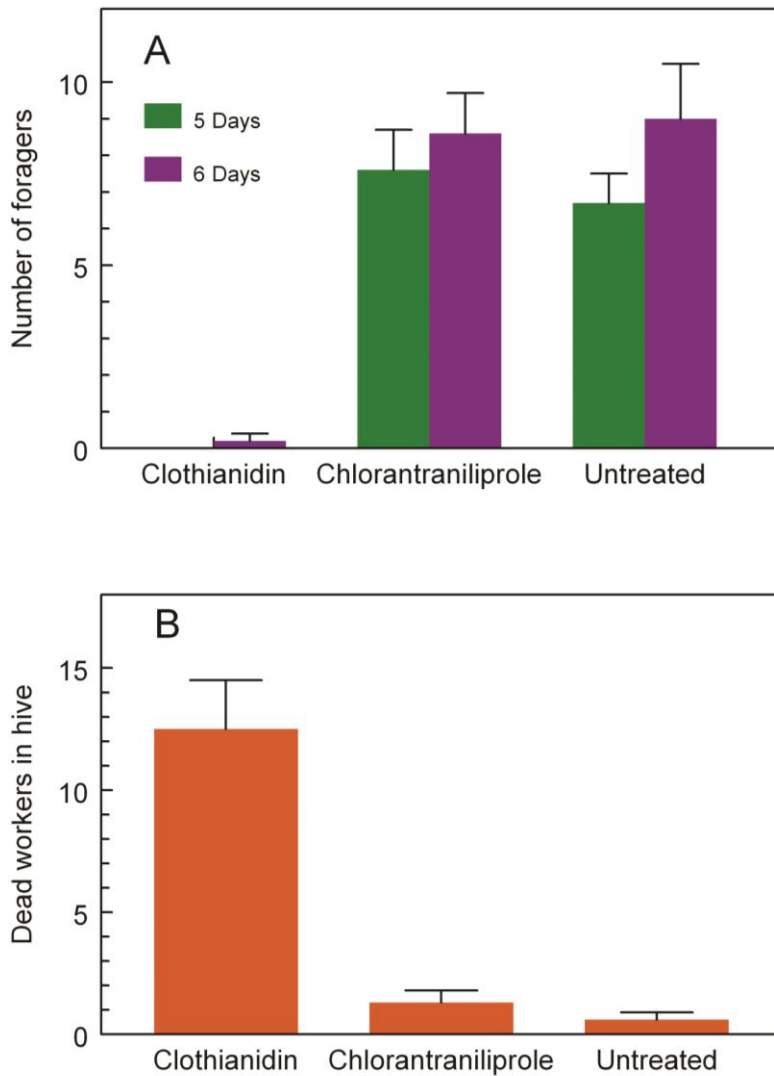


Figure 4.1. Foraging and dead workers during exposure to treated turf. Mean ( $\pm$ SE) numbers of (A) bees foraging in enclosures during two mid-afternoon inspections on the 5th and 6th days, and (B) dead non-callow workers observed in hives on the 6th day of exposure of bumble bee colonies to weedy lawn turf with residues of a neonicotinoid (clothianidin) or anthranilic diamide (chlorantraniliprole) applied at label rates.

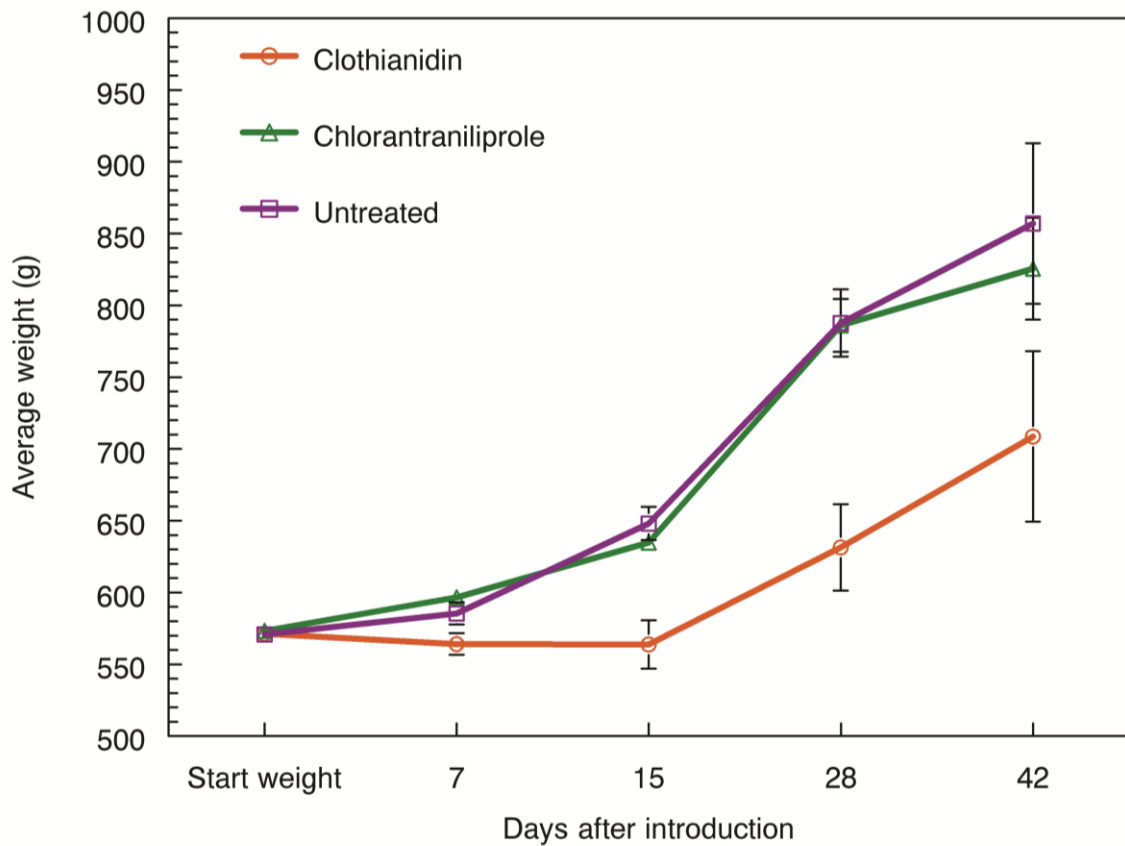


Figure 4.2. Colony weight change following exposure to treated turf. Mean (+ SE) weight change (g) of *Bombus impatiens* colonies (10 per treatment) after foraging 6 days on insecticide-treated lawn turf with white clover and then being moved to an insecticide free site to openly forage for another 6 weeks (Repeated measures ANOVA:  $F_{2,90} = 14.8$ ,  $P < 0.001$ ;  $F_{4,90} = 45.1$ ,  $P < 0.001$ ;  $F_{8,90} = 2.2$ ,  $P < 0.05$  for treatment, date, and treatment  $\times$  date interactions, respectively). Clothianidin-exposed colonies lagged behind the others on all dates ( $F_{2,18} = 6.5, 15.6, 12.7, 3.1$ ;  $P < 0.01, 0.001, 0.001, 0.07$  at 7, 15, 28, and 42 days after introduction, respectively).

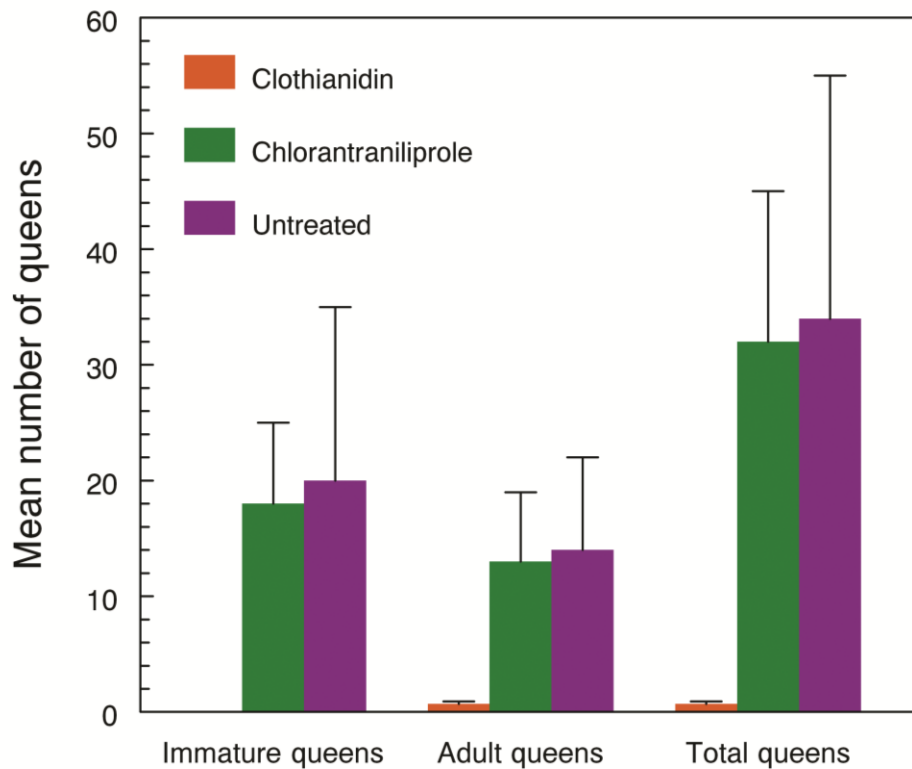


Figure 4.3. Queen production following exposure to treated turf. Mean (+ SE) numbers of queens produced by *Bombus impatiens* colonies that foraged for 6 days on insecticide-treated lawn turf with white clover and then were moved to an insecticide-free site to openly forage another 6 weeks (Friedman tests: Immature queens,  $P = 0.03$ ; Adult queens,  $P = 0.08$ ; Total queens,  $P = 0.05$ . Numbers of colonies (out of 10) that produced new queens were 0, 7, and 6 for clothianidin, chlorantraniliprole, and untreated hives, respectively. For the subset of colonies that produced new queens, those exposed to chlorantraniliprole-treated or untreated weedy turf produced similar numbers of immature, adult, and total queens (Kruskal-Wallis test,  $P = 0.69, 0.84, 0.95$ , respectively). Queens present in clothianidin exposed colonies likely represent the original mother queen.



Table 4.1 Condition of *Bombus impatiens* colonies that had been exposed to insecticide-treated turf with flowering white clover for 6 days, after which they were moved to an insecticide-free site to openly forage for 6 weeks before this evaluation.

Treatment	Adult bees per hive (excluding queens)				Immatures per hive <sup>a</sup>			
	Live	Dead	% dead	Total live wt (g)	Live	Dead	Honey pots	Hive wt (g)
Cloth. <sup>1</sup>	173 ± 39	33 ± 7	31.8 ± 11.1	28.2 ± 6.9	84 ± 15	9 ± 3	36 ± 12	709 ± 59
Chlor. <sup>2</sup>	199 ± 31	35 ± 14	17.4 ± 7.3	31.4 ± 4.8	45 ± 10	18 ± 9	51 ± 10	826 ± 35
Untreated	271 ± 30	54 ± 16	18.2 ± 5.9	42.9 ± 5.6	65 ± 14	27 ± 13	77 ± 22	857 ± 56

The turf was lightly irrigated after insecticide application; the surface had thoroughly dried before bees were introduced.

<sup>1</sup>= clothianidin, <sup>2</sup>= chlorantraniliprole

<sup>a</sup> Data are means (± SE). ANOVA (df = 2, 18): live, F = 2.31, P = 0.13; dead, F = 0.92, P = 0.42; % dead, F = 0.93, P = 0.41; wt live adults, F = 1.8, P = 0.19; live immature, F = 2.45, P = 0.12; dead immature, F = 0.90, P = 0.42, honey pots, F = 2.31, P = 0.13, hive wt, F = 2.27, P = 0.13. P-values from pre-planned linear contrasts between clothianidin versus untreated were 0.053, 0.23, 0.27, 0.28, 0.20, 0.09, 0.09, and 0.058, respectively. For chlorantraniliprole versus untreated, they were 0.15, 0.29, 0.95, 0.29, 0.51, 0.27, 0.18, and 0.67, respectively.

<sup>b</sup> larvae, pupae, and fully-formed workers and males still enclosed in the pupal exoskeleton within the cell.

<sup>c</sup> adult workers, males, and queens.

exposed to plots that had been treated with chlorantraniliprole showed no adverse effects compared to the untreated controls (Table 4.2). Notably, neither insecticide adversely affected a second set of colonies introduced into the enclosures after the turf had been mown to remove the original flower heads, and new flowers had formed (Table 4.3). Hives that had been confined on chlorantraniliprole-treated turf in fact had significantly higher numbers of live adult workers than did the untreated controls (two-tailed Dunnett's test,  $P = 0.02$ ; Table 4.3).

Neither bumble bees nor honey bees avoided foraging on white clover in turf that had been treated with either insecticide. Similar numbers of bumble bees, honey bees, and total bees were observed on clover blooms on each set of plots (Figure 4.4).

## **Discussion**

Field exposure to clothianidin, a representative from the neonicotinoid class, has the potential to impair queen production by bumble bee colonies foraging for less than a week on flowering weeds in recently-treated lawns. United States Environmental Protection Agency (EPA) label precautionary statements specify not to apply clothianidin, or other neonicotinoids, to blooming nectar-producing plants if bees are visiting the treatment area, but such exposures nevertheless may occur, especially when lawns are treated in spring for preventive grub control. Our results validate those EPA label precautions. They also confirm the results of other recent studies that showed acute mortality and impaired queen production when bees ingested neonicotinoid-spiked food (Whitehorn et al. 2012, Gill et al. 2012, Laycock et al. 2012), demonstrating similar effects from a plausible field exposure. Notably, no adverse effects were seen on bee

Table 4.2 Condition of *Bombus impatiens* colonies that were evaluated immediately after being exposed to insecticide-treated turf with flowering white clover for 2 wk.

Treatment	Adult bees per hive (excluding queens)			Immature bees per hive <sup>a</sup>		Honey pots	Hive weight (g)
	Live	Dead	Total live wt (g)	Live	Dead		
Cloth. <sup>1</sup>	59 ± 12*	26 ± 5*	7.7 ± 1.4*	21 ± 8	13 ± 2*	33 ± 5*	580 ± 17
Chloran. <sup>2</sup>	99 ± 12	6 ± 2	12.2 ± 1.5	31 ± 9	4 ± 1	47 ± 5	599 ± 11
Untreated	106 ± 8	7 ± 3	12.8 ± 1.6	17 ± 10	4 ± 1	51 ± 4	602 ± 6

<sup>a</sup>Includes larvae, pupae, and pharate adults

<sup>1</sup>= clothianidin, <sup>2</sup>= chlorantraniliprole

ANOVA (df = 2, 22): live,  $F = 4.57$ ,  $P < 0.05$ ; dead,  $F = 9.88$ ,  $P < 0.01$ ; wt live workers,  $F = 3.46$ ,  $P = 0.05$ ; live immature,  $F = 0.57$ ,  $P = 0.57$ ; dead immature,  $F = 9.25$ ,  $P < 0.01$ ; honey pots,  $F = 3.56$ ,  $P < 0.05$ ; hive wt,  $F = 0.69$ ,  $P = 0.51$ ; \*denotes means significantly higher or lower than colonies on untreated turf (Dunnett's test,  $\alpha = 0.05$ ).

Table 4.3 Absence of acute adverse effects on *Bombus impatiens* colonies after 2 weeks' exposure to turf with flowering white clover that had bloomed after the sward was mown to remove flowers present at the time of treatment.

Treatment	Adult bees per hive (excluding queens)			Immature bees per hive <sup>a</sup>		Honey pots	Hive wt (g)
	Live	Dead	Pooled live wt (g)	Live	Dead		
Cloth. <sup>1</sup>	93 ± 9	11 ± 4	13.0 ± 1.3	12 ± 8	6 ± 1	52 ± 6	585 ± 11
Chlor. <sup>2</sup>	130 ± 12	7 ± 2	16.7 ± 1.6	8 ± 4	6 ± 2	69 ± 6	621 ± 16
Untreated	81 ± 8	7 ± 2	11.3 ± 0.9	0	3 ± 1	56 ± 3	588 ± 8

Dates of insecticide application, mowing, and introduction of bee colonies were June 1, 15, and 22, respectively.

<sup>a</sup>Includes larvae, pupae, and pharate adults

<sup>1</sup>= clothianidin, <sup>2</sup>= chlorantraniliprole

ANOVA (df = 2, 19): live,  $F = 6.01$ ,  $P = 0.02$ ; dead,  $F = 1.05$ ,  $P = 0.37$ ; Wt live workers,  $F = 3.31$ ,  $P = 0.08$ ; live immature,  $F = 1.60$ ,  $P = 0.25$ ; dead immature,  $F = 0.54$ ,  $P = 0.6$ , honey pots,  $F = 2.15$ ,  $P = 0.17$ , hive wt,  $F = 1.93$ ,  $P = 0.20$

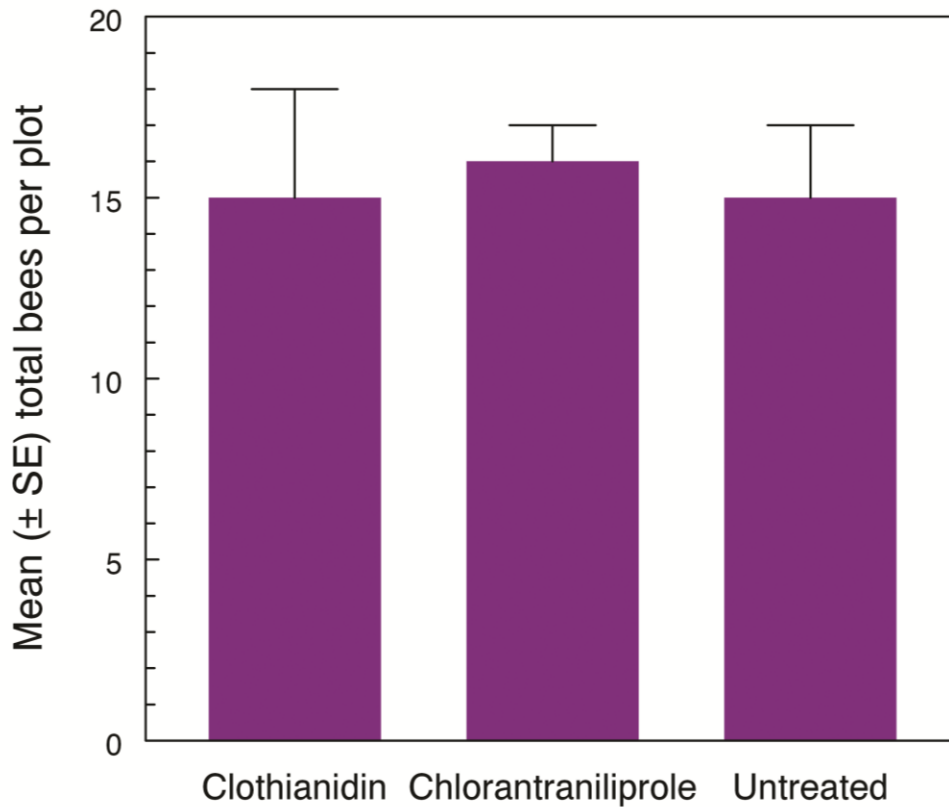


Figure 4.4. Non-avoidance of treated turf by bees. Bumble bees and honey bees did not discriminate between weedy lawn turf with or without non-irrigated insecticide residues ( $F_{2,8} = 0.02$ ,  $P = 0.98$ ). Plots were treated at label rate with residues left on the surface (not watered in) and a walk-through count of foragers on the intermixed white clover was taken on seven successive days. Data shown are mean ( $\pm$  SE) totals of both types of bees. Means for bumble bees were  $6.4 \pm 1.1$ ;  $6.6 \pm 0.9$ ,  $5.8 \pm 1.2$  ( $F_{2,8} = 0.16$ ,  $P = 0.85$ ); means for honey bees were  $8.8 \pm 1.5$ ,  $9.0 \pm 0.7$ ,  $9.4 \pm 0.5$  ( $F_{2,8} = 0.10$ ,  $P = 0.90$ ) for clothianidin-, chlorantraniliprole-, and non-treated plots, respectively.

colonies exposed to residues of chlorantraniliprole, a selective ryanodine receptor agonist, under the same conditions.

The concentrations of clothianidin we detected in clover nectar are higher than those that typically occur from systemic transfer of neonicotinoids into nectar of seed-treated crops (Hopwood et al. 2012, Bonmattin et al. 2003, 2005), and also much higher than lab-fed oral dosages of imidacloprid shown to adversely affect individual and colony-level traits, including reproduction, in bees (Mommaerts et al. 2010, Henry et al. 2012, Whitehorn et al. 2012, Gill et al. 2012, Laycock et al. 2012). A literature search found nothing on spatial or temporal translocation of neonicotinoids from roots into nectar or pollen of clover or similar small plants. Thus, while we can suggest several plausible ways that a lawn spray application might contaminate such nectar, the precise mechanisms by which it occurred in our study remain largely unknown.

The equipment with which we applied the insecticides, a lawn care spray gun and a multiple-nozzle boom sprayer, delivered similar pressure, droplet size, and spray volume as sprayers used in the turf care industry. It is likely that the sprays directly contaminated the nectar, which in non-pollinated *T. repens* florets is retained at the floret base for at least a week with no decrease in quantity or sugar content until pollination or senescence (Jakobsen and Kristjansson 1994). Clothianidin may also have been systemically translocated through foliage. Also, the numerous densely-arranged individual florets of not-yet-opened flower heads may have sufficient surface area shielded from UV light to allow translocation through cells of the nectary walls before such residues deteriorate. Although the turf was irrigated immediately after the insecticides were applied, some residues may have remained on the clover petals and

leaves, and on the turfgrass, so that foraging bees were exposed both through contact and ingestion.

Neonicotinoids are mainly acropetally transported in the xylem (Bucholz and Nauen 2001, Bonmatin et al. 2005, Blacquière 2012). Given clothianidin's prolonged (> 9 month) half-life from field dissipation in soil (US EPA 2003), it is unlikely that, in just three weeks, degradation of residues in the root zone can explain the lack of acute effects on bees foraging on clover that bloomed after mowing. Clothianidin is the least water-soluble neonicotinoid used on turf (US EPA 2003). Sorption of neonicotinoids to soil organic components reduces the amount that is translocated (Bucholz and Nauen 2001, Byrne et al. 2012). Neonicotinoid uptake via roots typically deposits the highest concentrations in the oldest foliage, with limited mobilization from mature to new leaves (Bucholz and Nauen 2001, Byrne et al. 2012), so in a mixed stand of turfgrass and flowering weeds, the competing grass could possibly act as a sink until being removed by mowing.

Clearly, more needs to be known about the movement and longevity of surface-applied neonicotinoids in clover and other small flowering plants to better interpret our results. Nevertheless, the results of our trial in which colonies were confined on treated weedy turf before or after the stand had been mowed, and earlier work showing absence of acute effects on bumble bees when a granular formulation of imidacloprid was applied to weedy turf and watered in (Gels et al. 2002), suggest that once the residues are leached into the soil by watering or rainfall, translocation via the roots is unlikely to pose a prolonged systemic hazard to bees.

Neither bumble bees nor honey bees avoided foraging on flowering clover contaminated with residues of clothianidin or chlorantraniliprole. That finding is consistent with previous studies showing bumble bees' non-avoidance of flowering clover in lawn grass that had been sprayed with imidacloprid (Gels et al. 2002), and bees' ready ingestion of syrup or plant guttation water containing toxic levels of neonicotinoids (Girolami et al. 2010, Gill et al. 2012). Thus, worker bees from colonies in non-treated landscapes may be exposed to insecticide residues when foraging on treated lawns. If such bees acquire a lethal dose they will not return to the colony, reducing its workforce. Even sublethal neonicotinoid exposure can impair workers' foraging efficiency, leading to food shortage and decreased colony success (Gill et al. 2012). Workers that bring contaminated nectar or pollen back to the colony could potentially affect development and survival of nest-mates. Bumble bee colonies are annual and only the new queens produced will survive the winter. In the spring, when queens are foraging and subsequently when colonies are small and contain only a few workers, they may be especially vulnerable to insecticide exposure (Goulson et al. 2008, Gill et al. 2012). Typically only the largest colonies succeed in producing queens (Müller and Schmid-Hempel 1992, Schmid-Hempel 2001, Gerloff and Schmid-Hempel).

It is possible, had we not sacrificed them, that clothianidin-exposed colonies could have recovered from the initial stress and produced queens later in the summer or autumn. However, any delay in switching from worker to queen production increases the chances of colony failure due to pathogens, predators, weather-related stress, or other factors. Moreover, queens produced later in the growing season are less likely to survive than are earlier-produced queens (Müller and Schmid-Hempel 1992, Schmid-Hempel



2001, Gerloff and Schmid-Hempel). Without timely investment in reproductive output, the potential loss of queen production due to neonicotinoid exposure could lead to lower local populations of bumble bees over successive years.

Besides mowing to remove flower heads before or immediately after application, bee exposure to pesticide residues on lawns could be reduced by controlling flowering weeds with herbicides or by delaying applications until after bloom of spring-flowering weeds. Such practices, however, may be difficult to ensure or may not always be practical, especially in high-volume commercial lawn care (Held and Potter 2012).

Anthranilic diamides, including chlorantraniliprole, show high selectivity for insect ryanodine receptors (RyRs) when compared to mammalian RyRs (Cordova et al. 2006, Wang et al. 2012). Chlorantraniliprole is active against caterpillars and some dipteran and coleopteran pests, mainly by ingestion and secondarily by contact (Cordova et al. 2006, Brugger et al. 2010). It appears to have little or no activity against predatory, parasitic, and social wasps, solitary and social bees, and ants (Cordova et al. 2006, Brugger et al. 2010, Larson et al. 2012). The basis for that selectivity is not yet understood but may involve differences in channel properties between RyRs of sensitive species and those of the aforementioned types of Hymenoptera (Wang et al. 2012).

Bumble bees and other native bees provide pollination services to urban and suburban gardens and landscapes (Tomasi et al. 2004, Goulson et al. 2008, Hernandez et al. 2009, Cameron et al. 2011, Hopwood et al. 2012). With their populations imperiled by habitat loss, diseases, parasites, and other stresses, reducing hazards posed to them by insecticides is important (Goulson et al. 2008, Hernandez et al. 2009, Potts et al. 2010,

Cameron et al. 2011, Hopwood et al. 2012). When neonicotinoids are applied to lawns, systemic hazard to bees through flowering weeds appears to be transitory and direct hazard can be mitigated by strict adherence to label precautions, or if blooms inadvertently are contaminated, by mowing to remove them. Chlorantraniliprole appears to be a good fit for industry initiatives to reduce the impacts of turf and landscape management on pollinators.

## CHAPTER FIVE

### **Mowing Mitigates Bioactivity of Neonicotinoid Insecticides in Nectar of Flowering Lawn Weeds and Turfgrass Guttation**

#### **Introduction**

Neonicotinoids are systemic insecticides that are potent selective agonists of nicotinic acetylcholine receptors in insects (Tomizawa and Casida 2003). They are commonly used on lawns, golf courses, and sports fields to manage pests that diminish aesthetics and suitability for play (Blaine et al. 2012; Held and Potter 2012). Applied as sprays or granules in spring, and watered in by irrigation or rainfall, they provide several months' residual control of root-feeding beetle grubs. Neonicotinoids are translocated acropetally in xylem to control some stem- and foliage-feeding pests (Held and Potter 2012). However, translocation from soil may also occur to nectar, pollen, or guttation of treated plants, which are ingested by bees or other beneficial insects (Shawki et al. 2005, Larson et al. 2013).

Label precautions specify not to apply neonicotinoids or allow them to drift to blooming plants if bees are visiting the area. In practice, however, flowering lawn weeds may be inadvertently over-sprayed when lawns or other turf areas are treated in spring, or they may sprout and bloom some weeks after the insecticide is applied (Larson et al. 2014). Ubiquitous weeds such as white clover (*Trifolium repens* L.) and dandelions (*Taraxacum officinale* Wigg) are attractive to native pollinators including bumble bees, solitary bees, and syrphid flies, as well as managed and feral honey bees (Larson et al. 2013). When turf intermixed with spring-flowering white clover was treated with

clothianidin, bumble bee colonies foraging on the sprayed flowers had higher worker mortality, reduced colony growth, and ultimately failed to produce new queens (Larson et al. 2013, 2014). In contrast, colonies exposed to treated turf after the directly sprayed blooms had been mowed from the plants, and new blooms had formed, showed no adverse effects. Bumble bee colonies foraging on blooming white clover in turf sprayed with imidacloprid also showed acute adverse effects, but a granular application had no measured impact on colony vitality (Gels et al. 2002). Although those findings suggest that soil-applied neonicotinoids are not translocated into flowering lawn weeds at high enough levels to pose a prolonged hazard to bees, little is known about their movement and longevity in such plants.

Guttation is the exuding of droplets of xylem sap from plant hydathodes on the tips and edges of leaves. Guttation regularly occurs in many different types of plants but is particularly notable in grasses (Williams et al. 1998, Girolami et al. 2009). It is often misconstrued as dew accumulating on grass blades and is crepuscular (Williams et al. 1998). Guttation droplets may fall, evaporate, or be absorbed back into the plant (Chen and Chen 2007), or bees and other beneficial insects may imbibe them (Shawki et al. 2006). Soil applications and seed treatments can result in neonicotinoid residues in the guttation of corn and melons, posing a possible hazard to bees (Girolami et al. 2009, Hoffman and Castle 2012), but such transference has not been assessed for turfgrass.

The extent to which translocated neonicotinoids impact bees or other beneficial insects in field settings is controversial and requires studies with relevant exposure and duration to resolve (Blacqui re et al. 2012). Turfgrasses cover about 164,000 km<sup>2</sup> in the continental United States, an area three times larger than that of any irrigated crop (Milesi

et al. 2005), so assessing the potential hazards of turfgrass insecticides to bees and other beneficial insects is important. In this study, LC-MS mass spectrometry was used to assess concentrations of imidacloprid or clothianidin in nectar from white clover blooms present in lawn grass when the turf was sprayed, and in new blooms formed soon after the first mowing. We also measured imidacloprid transference into guttation fluid of creeping bentgrass (*Agrostis stolonifera* L.) leaf blades. Bioactivity was determined by feeding the nectar or guttation to *Orius insidiosus* (Say), a small anthocorid bug that preys on grass-feeding caterpillars and other pests in turf (Joseph and Braman 2009). Our findings indicate that once the insecticide has been watered in and the turf is mowed, systemic transfer of residues into nectar of lawn weeds is negligible. Guttation fluid from treated turfgrass may pose a transitory hazard that is likely to be diluted by dew and irrigation.

## **Materials and methods**

### **Measurements of residues levels in clover nectar**

The experimental site was a mixed sward of Kentucky bluegrass (*Poa pratensis* L.) and turf-type tall fescue (*Festuca arundinacea* Schreb). with about 30% cover (by visual estimate) of flowering white clover on Maury loam soil (fine silty, mixed mesic type Paleudalf. pH 6.3) at the A.J. Powell Turf Research Center (AJPTRC), University of Kentucky, near Lexington, KY. It had been maintained as low-maintenance lawn grass; i.e., mowed weekly at 10 cm, and irrigated only as needed to alleviate visible drought stress. The turf was fertilized (75.6 kg N/ha from urea) once per year in November, and was not treated with insecticides until our treatments.

Plots ( $3.35 \times 3.35$  m) were treated with either imidacloprid (Merit 75 WSP; Bayer, Research Triangle Park, NC) or clothianidin (Arena 50 WDG; Valent, Walnut Creek, CA) at the insecticides' high label rates for preventive grub control (0.6 and 0.45 kg AI ha<sup>-1</sup>, respectively), or left untreated (no input check). The trial was repeated twice with the first set of plots (four replicates) treated on 3 June 2013 and the second set (two replicates) treated on 15 August 2013. We used a portable CO<sub>2</sub> spray tank (R and D Sprayers, Opelousas, LA) equipped with a 1.8 m handheld boom with four Spraying System 8004 Tee Jet nozzles (Spraying Systems, Wheaton, IL) that delivered a pressure of 2109 g cm<sup>-2</sup>. Spray volume was 468 L ha<sup>-1</sup>, applied by making two passes in opposite directions over each plot. Applications were lightly watered in (30.3 liters per plot) with watering cans. Separate spray bottles were used for each insecticide, and the spray apparatus was disassembled and cleaned between treatments. Residues were allowed to dry overnight and the next morning open-bottom screened enclosures (3.05×3.05 m; Instant Screen Shelters, Coleman Lantern; Wichita, KS) were erected on each plot to ensure bees did not collect nectar.

Three hundred open white clover flowers were hand-collected from each plot one day after the turf was sprayed (4 June and 16 August). Those directly-contaminated blooms were put into plastic bags and kept in a chilled picnic cooler before being placed in 4° C cold room. The plots were then immediately mowed (7 cm height), using a different push mower for each treatment to avoid cross-contamination. The screened enclosures were left on the turf until the intermixed white clover had produced enough new blooms for a second sample of 300 flowers per plot. Those samples, collected 17

June and 26 August (13 or 10 days after the first and second spray timings, respectively), were handled in the same manner as described above.

Nectar extractions took place over the 36-48 h after each flower collection. Individual flowers were suspended upside down inside 15mL Falcon centrifuge tubes (Thermo Fisher Scientific; Waltham, MA) with the stems folded across the lip as lids were screwed on to hold the flowers in place. The tubes were placed in a centrifuge (IEC Model HN SII Centrifuge; International Equipment; Needham Heights, MA) and spun for 10 min at 2200 rpm. Nectar from flowers within each plot was consolidated into micro-centrifuge tubes using a 200 mL pipette. Nectar was kept frozen until being taken to the University of Kentucky Environmental Research Training Laboratory (UK ERTL) for extraction and analysis. Nectar was extracted through Oasis HLB 1 cc Vac RC Cartridges (Waters Corporation; Milford, MA). The extracts were analyzed by liquid chromatography separation interfaced via electrospray ionization (ESI +) to a tandem mass selective detection system (LC-MS/MS) at 1 ppb level of detection. The LC system consisted of two Varian ProStar model 210 pumps (Varian; Palo Alto, CA), the LC column was a 150 x 2.00 mm Phenomenex Gemini C18 (Phenomenex; Torrence, CA) and the mass spectrometer was a Varian 1200L triple quadrupole. Before analysis, authenticated standards of each insecticide, provided by Bayer CropSciences, were used to create calibration curves. To ensure quality control, isotopically labeled internal standards were included in all samples and blank samples as well as spiked field samples.

#### **Measurement of imidacloprid transfer into turf guttation droplets**

Transference of imidacloprid into turfgrass guttation fluid was assessed in greenhouse and field trials. For the greenhouse trial, 80 cores (15.2 cm wide, 15 cm

deep) of turf and soil were pulled from an established stand of creeping bentgrass at the aforementioned AJPTRC on 30 October 2013. The bentgrass, maintained as a golf course fairway, was mowed 3 days per week at 1.6 cm, irrigated every other day with a sprinkler system, and fertilized with 146.5 kg/ha nitrogen per year from urea.

The cores were placed in pots on benches in a greenhouse and maintained at about 24 °C under ambient light supplemented with illumination (14:10 h [L/D] photoperiod) from sodium vapor lights. A handheld trigger-type sprayer head with the siphon tube inserted into a 15 mL centrifuge tube containing the insecticide solution was used to apply imidacloprid at the aforementioned high label rate, followed by light watering (35 mL per core) to move the residues into the soil. There were 40 treated cores, the remainder serving as untreated checks. The cores were irrigated with about 100 mL of irrigation from a water hose with a shower-type nozzle and clipped (7 cm) with hand shears every other day.

To assess imidacloprid movement into guttation of field-grown turfgrass, plots ( $3.4 \times 3.4$  m) in the aforementioned creeping bentgrass sward were treated with imidacloprid in the same manner as described for the trials with clover nectar. There were five replicates each of treated and untreated (no-input check) plots. The sprays were applied on 30 May 2013, followed immediately by irrigation (30.3 liters per plot) from watering cans. The field plots were irrigated daily by an automated sprinkler system, and mowed 3 days a week with a reel mower (1.6 cm cutting height) over the next 3 weeks to simulate typical golf course fairway maintenance. The night before guttation droplets were collected,  $3.05 \times 3.05$  m open-bottom screened enclosures of the



type described earlier were erected over each plot to reduce accumulation of atmospheric condensation on the turfgrass.

Guttation fluid was collected at 1 and 3 weeks after treatment in each trial. Collections were made between 8:00 and 10:00 a.m. A specialized glass pipette (Williams et al 1998) was hooked to a vacuum pump and used to collect individual guttation droplets, by replicate, from the tips of grass blades within each core or plot. The sampling continued until at least 1.5 mL of guttation fluid water had been collected per replicate. Collected guttation was transferred to micro-centrifuge tubes, frozen, and taken to the UK ERTL lab where imidacloprid content was assessed by the same LC-MS/MS methods used for the clover nectar.

### **Toxicity trials with *Orius insidiosus***

Adult *O. insidiosus* were used to test bioactivity of both types of plant exudates following insecticide treatment. The bugs were obtained from ARBICO Organics (Oro Valley, AZ). Petri dishes ( $6.5 \times 1.5$ cm) with no substrate were each provisioned with three 5  $\mu$ L droplets of nectar or guttation fluid about 1 cm apart in a triangular array. To replicate the experiment, nectar or guttation from multiple field plots was used when setting up ten different petri dishes with *Orius*. In addition to nectar or guttation water from treated or untreated plots, a positive control (application-ready imidacloprid tank mix, to confirm insecticide activity), and a negative control (Cool Blue Gatorade; PepsiCo; Purchase, NY; to confirm assay suitability for the bugs), were included. Each dish was provisioned with 10 adult *O. insidiosus* which were observed to readily imbibe fluid from the droplets. Dishes with insects and droplets were placed in a growth chamber

(Percival Electric; Perry, IA) set at 25°C in darkness. Numbers of still-living (showing any movement at all) bugs were counted after 24 h. To assess sublethal motor impairment, still-living individuals were placed in the center of circle (8 cm diameter) drawn on paper and given 1 minute to walk beyond the edge.

### **Statistical analyses**

Data were analyzed within each trial using analysis of variance (ANOVA) for completely randomized (residue analysis) or randomized complete block (RCB) designs (*Orius* trials). Data were log-transformed when needed to correct for heterogeneity of variance. Mean separation in the *Orius* feeding trials was by Fisher's least significant difference (LSD) test at  $P \leq 0.05$ . Analyses were done with Statistix 9.0 (Analytical Software 2013). Data are presented as original means  $\pm$  standard error (SE).

### **Results**

#### **Nectar analyses and toxicity to *Orius insidiosus***

Insecticide levels in nectar from white clover blooms directly present during spraying ranged from 3281–7817 ng/g for imidacloprid, and from 1883–4475 ng/g for clothianidin (Table 5.1). Nectar from both of those treatments was toxic to *O. insidiosus* (2-way RCB ANOVA,  $F_{4, 12} \geq 6.38$ ,  $P \leq 0.005$ ; Figure 5.1) and comparable to the >90% mortality of bugs provisioned with droplets of the imidacloprid tank mix. Nectar from white clover in non-treated turf did not contain detectable residues of either insecticide. Mortality of bugs fed clover nectar from non-treated plots (Figure 5.1) or Gatorade (data not shown) was negligible.

**Table 5.1** Insecticide residue levels found in two trials (spring and fall) of white clover nectar directly contaminated with either imidacloprid or clothianidin and levels of the same insecticides in new flowers from the same plots after mowing.

Application time	Imidacloprid (ng/g)		Clothianidin (ng/g)	
	Direct Contamination	Post Mowing	Direct Contamination	Post Mowing
Spring	5493 $\pm$ 1040	8.4 $\pm$ 2.2	2992 $\pm$ 541	6.2 $\pm$ 2.1
Fall	6588 $\pm$ 752	26 $\pm$ 10	2882 $\pm$ 228	18 $\pm$ 15

Statistical analysis: One way ANOVA between direct contamination and post mowed nectar; Imidacloprid, spring:  $F_{1,7} = 27.9$ ,  $P = 0.002$ ; fall  $F_{1,4} = 136.9$ ,  $P = 0.001$ ; Clothianidin, spring:  $F_{1,7} = 30.6$ ,  $P = 0.002$ ; fall  $F_{1,4} = 281.0$ ,  $P = 0.0005$

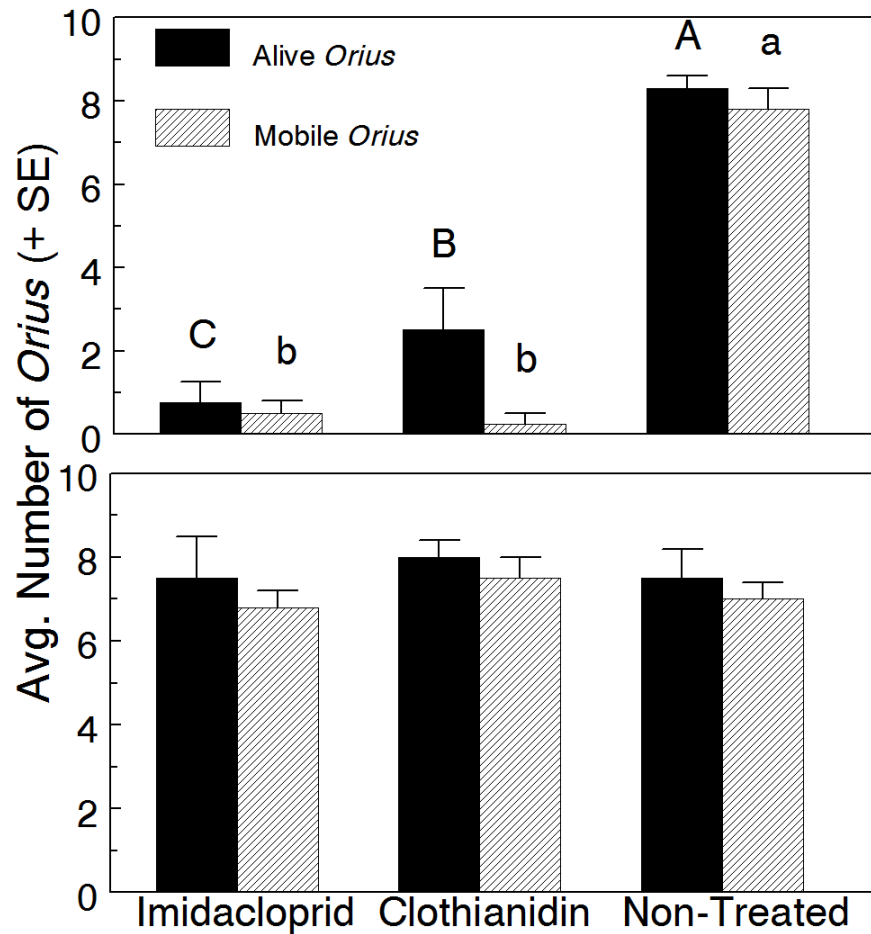


Figure 5.1. *Orius* feeding trials showing toxicity of nectar from directly-contaminated white clover flowers in neonicotinoid-treated turf (upper graph), and absence of effects from imbibing nectar from new blooms formed soon after the turf was mowed (lower graph). Bars (means  $\pm$  SE) within alive or still-mobile groups not topped by the same letter differ significantly (ANOVA, LSD,  $P \leq 0.05$ ).

Residue levels in nectar from new blooms that opened after the turf had been mowed were reduced by 99.4–99.8% compared to levels in directly-sprayed blooms (Table 5.1). There were no significant lethal or sub-lethal effects on *Orius* feeding on nectar from post mowed plots when compared to controls (RCB ANOVA,  $F_{3,9} \leq 0.67$ ,  $P \geq 0.59$ ; Figure 5.1).

### **Guttation analysis and *Orius* feeding assay**

Imidacloprid residues in guttation fluid from imidacloprid-treated creeping bentgrass were much higher in the greenhouse than in the field (Table 5.2). Those concentrations significantly declined, by about 88% in the greenhouse and 74% in the field, between 1 and 3 weeks post-treatment. Guttation fluid from non-treated turf did not contain detectable imidacloprid.

*Orius insidiosus* that imbibed creeping bentgrass guttation fluid collected in the greenhouse at 1 or 3 weeks after treatment suffered significant mortality compared to bugs provided guttation droplets from non-treated grass (Fig 5.2;  $F_{5,45} \geq 10.7$ ,  $P < 0.001$ , linear contrasts within dates). In contrast, bugs provided field-collected creeping bentgrass guttation fluid had similarly high survival regardless of whether the exudate came from treated or non-treated turf ( $F_{5,45} \leq 1.47$ ,  $P \geq 0.23$ ). As before, there was little or no mortality of bugs fed Gatorade, and nearly complete mortality of those provided droplets of the imidacloprid tank mix (data not shown).

### **Discussion**

This study evaluated two previously unstudied modes of exposure by which translocated neonicotinoid residues might intoxicate non-target insects in turfgrass

**Table 5.2** Imidacloprid residue levels found in two trials, one in the field and one in the greenhouse, guttation droplets collected from creeping bentgrass (*Agrostis stolonifera* L.) at one and three weeks after treatment.

Collection site	1 week after treatment (ng/g) <sup>a</sup>	3 weeks after treatment (ng/g)
Greenhouse	7050 ± 1107	820 ± 99
Field	88 ± 35	23 ± 3

<sup>a</sup>Statistical analysis: One way ANOVA between imidacloprid at one and three weeks; Field:  $F_{1,9} = 11.64$ ,  $P = 0.009$ ; Greenhouse:  $F_{1,7} = 31.43$ ,  $P = 0.001$ .

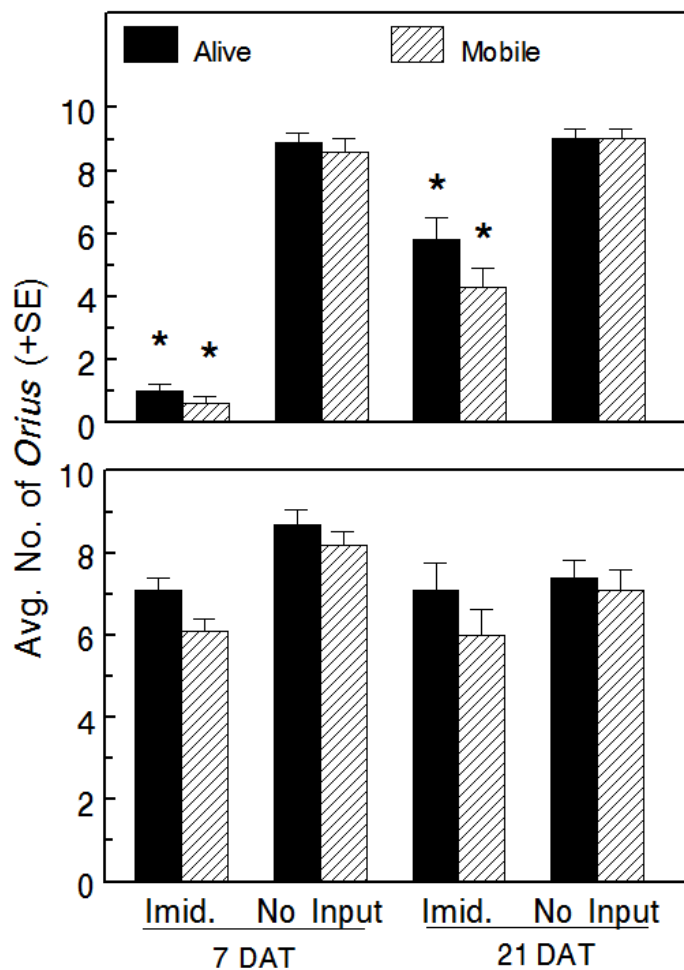


Figure 5.2. *Orius* feeding trials using guttation droplets collected from imidacloprid (Imid.) or non-treated creeping bentgrass. Upper graph shows effect of feeding on guttation droplets collected in a greenhouse, lower graph guttation droplets collected from bentgrass in the field. Bars marked with an \* differ significantly from check, see text for statistical comparisons.

settings. Not surprisingly, concentrations of imidacloprid and clothianidin detected in nectar from clover blooms that were oversprayed when the turf was treated were much higher than what typically occurs from systemic transfer nectar of seed-treated crops (Bonmatin 2003, 2005, Hopwood et al. 2012). Nectar in non-pollinated *T. repens* florets is retained at the floret base for at least a week with no decrease in quantity or sugar content until pollination or senescence (Jakobsen and Kristjánsson 1994). The numerous densely-arranged individual florets of not-yet-opened flower heads may have sufficient surface area shielded from UV light to allow translocation through cells of the nectary walls before such residues deteriorate. Therefore if blooming lawn weeds are inadvertently oversprayed with neonicotinoids in violation of label precautions, bees and other nectar feeders could be intoxicated. Indeed, bumble bee colonies that foraged on turf with flowering white clover that had been directly sprayed with clothianidin showed reduced foraging and delayed weight gain compared to control colonies, and produced no new queens (Larson et al. 2013).

White clover and other lawn weeds can be difficult to completely eliminate with herbicides (Ricigliano 2013) so weeds may be inadvertently oversprayed when homeowners or lawn care providers apply preventive grub treatments in spring. Some lawn care companies tank-mix a post-emergent herbicide and neonicotinoid to save fuel, time, and labor. In such cases, bees and other pollinators could be exposed to spray residues on, or in, the blooms and nectar before the weeds are controlled. For example, neither bumble bees nor honey bees avoided foraging on clothianidin-treated white clover in open plots (Larson et al. 2013). Weeds may also germinate and flower some weeks after neonicotinoids are applied, either in spring, or for white clover, also in late summer



when neonicotinoids may be applied to control insect pests already present in the soil. It therefore was relevant to assess the extent of translocation from the soil into nectar of blooms formed after treatment and the first mowing. Our results indicate that residue levels in blooms formed after a single mowing were reduced by 99.4–99.8% compared to levels in directly sprayed blooms. Those trace levels were not toxic to *Orius*. Similarly, bumble bee colonies exposed to new clover flowers in clothianidin-treated lawn turf exhibited no negative effects (Larson et al. 2013).

Neonicotinoids are mainly acropetally transported in the xylem (Bucholz and Nauen 2001, Bonmatin 2005, Blacquière et al. 2012). Translocation is driven by transpiration and plant growth processes likely to be greater for turfgrass foliage than for floral tissues and nectar of blooming weeds. Neonicotinoid uptake via roots typically deposits the highest concentrations in the oldest foliage, with limited mobilization from mature to new leaves (Bucholaz and Nauen 2001, Byrne et al. 2012). Therefore, in a mixed stand of turfgrass and flowering weeds, the competing grass may act as a sink until being removed by mowing. Given the prolonged ( $\geq 9$  month) half-life of neonicotinoid insecticides in soil (US EPA 2003, Gervais et al. 2010) and their residual effectiveness against soil insects, it is unlikely that residues in the root zone had degraded enough in the 10-13 days between treatment, mowing, and formation of new flowers to explain the aforementioned results. Our findings suggest that once they are watered in and the turf has been mowed, neonicotinoid residues in weed nectar or guttation fluid are unlikely to pose a prolonged systemic hazard to beneficial insects.

Guttation fluid may serve as a source of water for bees and other non-target invertebrates (Thompson 2010, Pistroius et al. 2012). Indeed, we observed ants and flies

imbibing the droplets during our field collections. Periods with cool nights and mornings followed by warm days such as occur in spring are particularly conducive to guttation (Thompson 2010). In our trials, neonicotinoid levels in field-collected creeping bentgrass guttation fluid declined by about 74% between 1 and 3 weeks after treatment, and were not toxic to *Orius*. Similarly, studies with neonicotinoid seed-treated crop plants indicate that peak residue levels occur at the onset of guttation in young plants and decline over time (Pistorius et al. 2012).

Levels of neonicotinoid residues in guttation fluid differed greatly between field collected and greenhouse collected samples. In the field, guttation droplets were probably diluted by mixing with moisture from atmospheric condensation (Williams et al. 1998) despite our efforts to reduce dew formation by placing screened enclosures on the plots on nights before guttation was collected. In the greenhouse, with more control over the environment, atmospheric condensation was not present during droplet collections. In the greenhouse, guttation was only produced when the turfgrass was covered and heavily watered the preceding day. Because the field-grown turf received greater amounts of irrigation from an automated sprinkler system and was exposed to cool nights and warmer days, it likely expelled guttation droplets daily, with lower insecticide concentrations each time. Future studies on insecticide translocation in guttation fluid, regardless of the plant system involved, should not assume that concentrations in guttation from greenhouse or lab grown plants are indicative of those in the field

Increasing awareness of the potential impacts of insecticides on bees and other non-target species is helping fuel an industry-wide shift toward more environmentally-benign lawn care practices, one that will inform business decisions in the future.

Flowering lawn weeds attract diverse pollinator assemblages in cities and suburbs (Larson et al. 2014). Those plants, and turfgrass guttation fluid, also help sustain natural enemies that provide biological control services (Braman et al. 2002, Dobbs and Potter 2014). With an estimated 84 million households engaged in lawn and garden activities (US-EPA 2012), and golf courses around the world implementing pollinator conservation initiatives (Dobbs and Potter 2013) protocols for bee-friendly turf care are needed.

Our results indicate that systemic residues in nectar of flowering lawn weeds decrease drastically after mowing. Systemic residues are also relatively low in guttation droplets and in field settings they will be diluted by dew or irrigation. The sugar levels in guttation fluid are too low to be attractive to bees as a nectar replacement (Thompson 2010). Bees may collect guttation as one of many water sources in the surroundings of a colony, but it only available for a short period in early morning (Pistorius et al. 2012). With adherence to label precautions, mowing, and irrigation, hazard of neonicotinoids to insects that forage on nectar or guttation water in turf can be greatly reduced.

## **CHAPTER SIX**

### **Pollinator Diversity on Dandelions and White Clover in Urban and Suburban Lawns**

#### **Introduction**

Removal and fragmentation of natural habitat due to human perturbations is a leading contributor to declining pollinator populations (Kearns et al. 1998, Biesmeijer et al. 2006, Goulson et al. 2008, Potts et al. 2010, Cameron et al. 2011). In the United States about one million hectares of farmland and natural habitat are converted to urban and suburban areas each year (McFredrick and Lebuhn 2006). As urbanized landscapes expand, it is important to understand their capacity to support native bees and other insects that provide pollination services within remaining fragments of natural habitat as well as to community and backyard gardens. Surveys indicating that suburban habitats may still harbor a high abundance and diversity of wild bees have mainly focused on pollinator assemblages in gardens (e.g., Frankie et al. 2005, Fretwell et al. 2008, Matteson et al. 2008, Ahrné et al. 2009) or used non-discriminate pan traps or sweep-netting from mixed, ruderal vegetation to compare pollinator assemblages across different habitat types (e.g., Tommasi et al. 2004, Bates et al. 2011). The potential role of low-input turf grass lawns in pollinator conservation is largely unstudied.

Turf grasses cover about 164,000 km<sup>2</sup> in the continental United States, an area three times larger than that of any irrigated crop (Milesi et al. 2005). More than 75% of that area is composed of tens of millions of residential, commercial, or institutional lawns; the rest is subdivided into golf courses, sport fields, playgrounds, parks,

cemeteries, sod farms, and other sites (Milesi et al. 2005, Held and Potter 2012). Lawns, especially relatively low-input ones, typically have varying densities of white clover (*Trifolium repens* L.), dandelions (*Taraxacum officinale* Wigg), or other flowering weeds that are attractive to bees and other pollinators (Larson et al. 2013). Though flowering weeds are often deemed undesirable and suppressed with herbicides, they may serve as an under-appreciated resource for resident pollinators. Documenting such associations could help foster a more ecological mindset wherein some flowering lawn weeds are tolerated, or even encouraged as a component of urban pollinator conservation efforts. It could also call attention to the types of pollinators that could be impacted by non-discriminate use of lawn insecticides (Larson and Potter 2013).

Every lawn has a unique surrounding landscape so it cannot be assumed that the pollinator assemblages of flowering weeds will be similar for lawns in urban, suburban, or periurban-rural areas. Surveys of pollinators in comparable habitats across gradients of increasing urbanization indicate that the surrounding landscape can affect pollinator abundance, species richness, and diversity (Ahrné et al. 2009, Hernandez et al. 2009, Bates et al. 2011, Banaszak-Cibicka and Żmihorski 2012). Although urban and suburban landscapes, with their higher amounts of manmade impervious surfaces (i.e. roads, parking lots, buildings), tend to support lower diversity of native plants than do more natural areas (Blair et al. 1997, Marzluff 2001), those that incorporate flower-rich green space can still harbor abundant and diverse populations of native bees and other pollinators (McKinney 2002, 2006; Cane et al. 2006, Winfree et al. 2009, Carré et al. 2009). As yet, no studies have compared how the nature of the surrounding landscape

affects pollinator assemblages in weedy lawns. Such information might help inform landscape design to support urban pollinator conservation.

Our objectives were to 1) characterize the pollinator assemblages visiting flowering dandelion and white clover in lawn type settings and compare them between those plant species, 2) compare species and diversity of pollinators visiting clover blooming in spring or late summer, and 3) test the hypothesis that species richness and diversity of pollinators on lawn weeds is negatively affected by increasing proportion of impervious surfaces surrounding lawn sites along an urban-rural gradient.

## **Materials and Methods**

### **Pollinator Assemblages of Flowering Dandelion and White Clover in Suburban Lawns**

Home lawns tend to be heterogeneous in their management and local habitat characteristics, so we sampled instead from flowering dandelions and white clover in large (0.46–1.07 ha) low-input lawns mostly in municipal parks, with one location each on the University of Kentucky campus and at University of Kentucky A.J. Powell, Jr. Turfgrass Research Center, Lexington, Kentucky. The lawns, mixed stands of mostly Kentucky bluegrass (*Poa pratensis* L.) and tall fescue (*Festuca arundinacea* Shreb.), were mowed at 5.1–8.4 cm every 7–10 days and received no irrigation or pesticides.

We sampled pollinators from each flower species at four different sites, separated by at least 1 km, in both 2011 and 2012. Dandelion, a predominantly spring-blooming plant, was sampled 25–27 May 2011, and 1–3 May 2012. White clover abundantly blooms both in spring and late summer so it was sampled twice in each year, 1–3 June

and 23–25 August in 2011, and 7-9 May and 15-17 August in 2012. Different sites were sampled in 2011 and 2012. At each site, an area ( $30 \times 30$  m) with abundant flowering plants of one or the other weed species was designated for sampling. All collections were done between 11 a.m. and 3 p.m. on clear days by slowly walking across the lawn and individually netting the first 50 pollinators encountered directly from the flower heads. Only those insects that alighted and remained on a flower head for  $\geq 3$  seconds were collected. Samples were immediately transferred to 70% EtOH for killing and preservation before pinning.

Most specimens were identified by the authors using keys and descriptions in Mitchell (1960, 1962) and Michener (2007). Some reference specimens, mainly Andrenidae, were sent to JS Ascher (American Museum of Natural History, New York, NY) who identified them.

### **Pollinator Assemblages of White Clover in Lawns across an Urban-Rural Gradient**

Eighteen mixed Kentucky bluegrass-tall fescue lawns with flowering white clover were sampled along a gradient extending from urban neighborhoods near the city center of Lexington, Kentucky (coordinates 38.047888,-84.500241) into suburban and periurban-rural areas so that the sites varied in the percentage of surrounding area covered by concrete and asphalt. Sixteen of the lawn sites were in municipal parks, one surrounded an elementary school, and one was at the University of Kentucky A.J. Powell, Jr. Turfgrass Research Center. The two outermost sites were 12 and 18 km from the city center. All of the lawns were mowed every 7-10 days, but received no irrigation or pesticide applications. The area of turf at each site ranged from 1900 to 11,500 m<sup>2</sup>.

None of the sampled lawn areas were bordered by gardens or contained flowering plants other than white clover and other less abundant lawn weeds.

Bumble bees foraging in fragmented habitats tend to show site constancy in relation to patchiness of floral resources, revisiting the same patches of forage day after day (Osborne and Williams 2001). Solitary bees, too, typically have a relatively small foraging range (150–600 m) with local habitat structure of more importance than large-scale landscape characteristics (Gathmann and Tschardt 2002). Therefore, to characterize the extent of urbanization of the landscape surrounding the sample sites at lawns at a relevant spatial scale, aerial images of each sample site from Google Earth (<http://www.google.com/earth/>) were overlaid with circles having radii of 200 and 500 m using KML4 Circle generator (<http://kml4earth.appspot.com/circlegen.html>). Areas with impervious surfaces within each circle were digitally colored-in using Microsoft Paint (<http://windows.microsoft.com/en-us/windows7/products/features/paint>); then ImageJ software (<http://rsb.info.nih.gov/ij/>) was used to calculate the percentage of impervious surface within each radius. For some analyses, the lawn sites were grouped into three categories: urban (7 sites), suburban (4 sites), or periurban-rural (7 sites) for which the percentage of impervious surface within a 200 m radius ranged from 20.2–34.6%, 15.4–16.8%, and 1.9–8.6%, respectively.

Pollinator collections were made on clear days between 11 a.m. and 3 p.m. over 18 days (7–25 May 2012) as described earlier; i.e., by slowly walking across the lawn and individually netting the first 50 pollinators encountered that alighted and remained on clover blooms for at least 3 seconds. Specimens were preserved and identified as previously described.



## **Data analyses**

Species richness and diversity (Simpsons 1-D, Magurran 2004) were calculated for the collections from each site and with collections from 2012, compared between clover and dandelions, and between spring and late-summer blooming clover, by Wilcoxon signed rank tests. Proportions of samples represented by honey bees (*Apis mellifera*), bumble bees (*Bombus* spp.), solitary bees (Andrenidae, Halictidae, Megachilidae), and flower flies (Syrphidae) were compared between weed species, and between clover sample dates, by chi-square tests on pooled samples from the four sites within each year, and for both years combined.

The hypothesis that pollinator species richness and diversity in lawns declines with increasing urbanization was tested for the 18 sites along the urban-rural gradient by linear regression, with percentage of impervious surfaces within a 200 m radius as the independent variable. The same hypothesis was similarly tested for number of honey bees or bumble bees in the 50-pollinator sample from each lawn. Percentages of samples comprised of honey bees, bumble bees, solitary bees, and Lepidoptera (butterflies and skippers) were compared between urban, suburban, and periurban-rural sites by one way analysis of variance (ANOVA) on arcsine square root-transformed data. Statistix 9.0 (Analytical Software 2013) was used for analyses. Data are presented as raw (non-transformed) means  $\pm$  standard error (SE).

## **Results**

### **2011 Clover-Dandelion Pollinator Collections**

In 2011 we collected and identified 629 individual insect pollinators from the flowering weeds in urban-suburban lawns representing 25 and 23 different species from dandelions and white clover, respectively. The most abundant taxa on spring-blooming dandelions included flower flies (Syrphidae), honey bees, chrysomelid beetles, and solitary bees, especially Halictidae and Andrenidae (Table 6.1). The predominant taxa visiting spring-blooming clover were honey bees, two solitary ground nesting bees, *Andrena wilkella* Kirby and *Calliopsis andreniformis* Smith (Andrenidae) and flower flies. Honey bees, the common eastern bumble bee *Bombus impatiens* Cresson, and several species of butterflies including the common buckeye butterfly *Junonia coenia* Hübner and fritillaries, *Speyeria* spp. (Nymphalidae) dominated the white clover pollinator assemblages in late summer (Table 6.1).

The proportions of social bees (*A. mellifera* and *Bombus* spp.) versus non-social bees (Andrenidae, Halictidae, Megachilidae) did not differ between clover and dandelion in the spring (Figure 6.1;  $\chi^2 = 1.66$ ,  $P = 0.23$  for pooled samples from four sites for each plant species). Social bees comprised a higher proportion of the pollinator assemblage of white clover in late summer than in spring (Figure 6.1;  $\chi^2 = 69.6$ ,  $P < 0.001$ ).

## **2012 Clover-Dandelion Collections**

A total of 634 individual pollinators were collected and identified from flowering lawn weeds in 2012, including 18 and 23 different species from dandelion and white clover, respectively (Table 6.2). Species richness on spring-blooming dandelions ranged from 3–7 species per lawn site. The number of species collected from clover ranged from 6–10 and 3–8 per lawn site for the spring and summer samples, respectively.

Table 6.1. Total numbers and the percentage of overall collection for each pollinator species captured on dandelion and clover flowers in 2011, categorized by tribe or family.

Pollinator species	Dandelion		Clover			
	Spring		Spring		Late summer	
	N	%	N	%	N	%
<b>Apini</b>						
<i>Apis mellifera</i>	48	22.2	91	44.2	137	66.2
<b>Bombini</b>						
<i>Bombus bimaculatus</i>	0	0.0	2	1.0	0	0.0
<i>B. griseocollis</i>	6	2.8	5	2.4	0	0.0
<i>B. impatiens</i>	3	1.4	7	3.4	12	5.8
<i>B. pennsylvanicus</i>	2	0.9	0	0.0	0	0.0
<b>Ceratini</b>						
<i>Ceratina calcarata</i>	0	0.0	1	0.5	0	0.0
<b>Nomadini</b>						
<i>Nomada articulata</i>	0	0.0	1	0.5	0	0.0
<i>Nomada imbricata</i>	1	0.5	0	0.0	0	0.0
<b>Andrenidae</b>						
<i>Andrena barbera</i>	2	0.9	0	0.0	0	0.0
<i>A. commada</i>	1	0.5	0	0.0	0	0.0
<i>A. crataegi</i>	1	0.5	0	0.0	0	0.0
<i>A. cressonii</i>	2	0.9	0	0.0	0	0.0
<i>A. illini</i>	1	0.5	0	0.0	0	0.0
<i>A. miserabilis</i>	2	0.9	0	0.0	0	0.0
<i>A. morrisonella</i>	10	4.6	0	0.0	0	0.0
<i>A. nasonii</i>	3	1.4	0	0.0	0	0.0
<i>A. perplexa</i>	2	0.9	0	0.0	0	0.0
<i>A. wilkella</i>	0	0.0	41	19.9	0	0.0
<i>Calliopsis andreniformis</i>	0	0.0	13	6.3	1	0.5
<b>Halictidae</b>						
<i>Agapostemon viscerans</i>	0	0.0	2	1.0	0	0.0
<i>Augocholorella aurata</i>	5	2.3	0	0.0	0	0.0
<i>Halictus c. confusus</i>	1	0.5	0	0.0	0	0.0
<i>H. ligatus</i>	15	6.9	3	1.5	0	0.0
<i>H. rubicundus</i>	1	0.5	3	1.5	0	0.0
<i>Lasioglossum sp.</i>	4	1.9	0	0.0	0	0.0
<b>Megachilidae</b>						
<i>Megachile rotunda</i>	1	0.5	4	1.9	0	0.0
<b>Sphecidae</b>						
<i>Sceliphron caementarium</i>	0	0.0	0	0.0	1	0.5
<i>Ammophila procera</i>	0	0.0	1	0.5	2	1.0
<b>Vespidae</b>						
<i>Polistes sp.</i>	1	0.5	0	0.0	0	0.0
<b>Lycaenidae</b>						
<i>Cupido commyntas</i>	0	0.0	4	1.9	9	4.3
<b>Hesperiidae</b>						
<i>Hesperia comma</i>	0	0.0	7	3.4	0	0.0

Table 6.1. Continued

**Nymphalidae**

<i>Junonia coenia</i>	0	0.0	0	0.0	27	13.0
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<i>Speyeria cybele</i>	0	0.0	0	0.0	1	0.5
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<i>Speyeria</i> sp.	1	0.5	5	2.4	12	5.8
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**Pieridae**

<i>Pontia protodice</i>	0	0.0	0	0.0	2	1.0
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<b>Chrysomelidae</b>	24	11.1	0	0.0	0	0.0
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<b>Canopidae</b>	1	0.5	1	0.5	0	0.0
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<b>Syrphidae</b>	78	36.1	15	7.3	3	1.4
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<b>TOTAL</b>	216		206		207	
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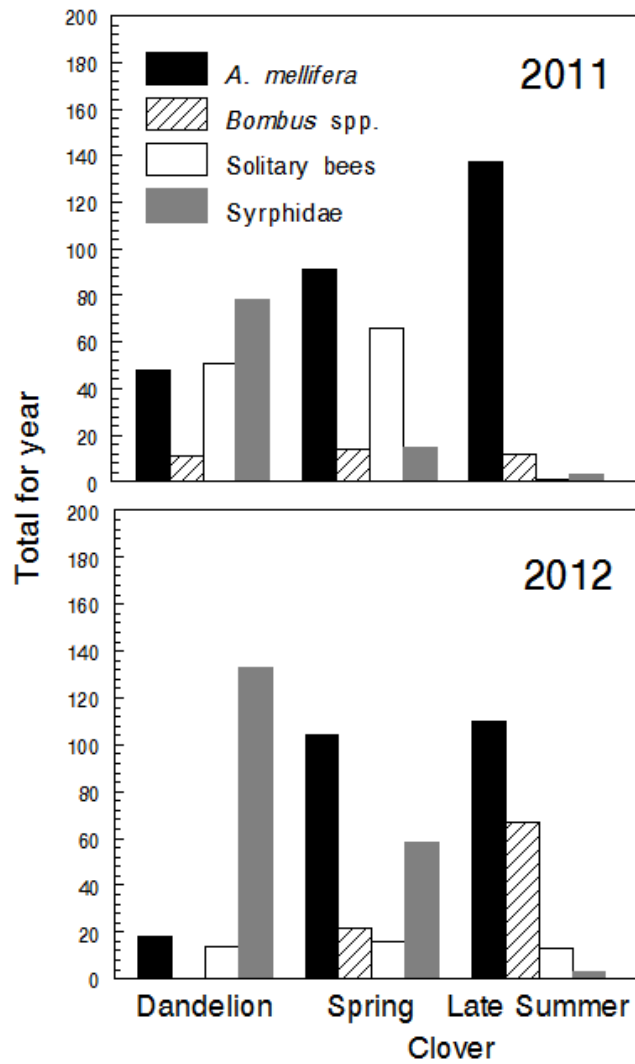


Figure 6.1. *A. mellifera*, *Bombus* spp., solitary bees, and syrphid flies collected from flowering dandelion in the spring and white clover in both spring and late summer. Total numbers of each group, pooled across sample sites, are shown for each year.

Table 6.2. Total numbers and the percentage of overall collection for each pollinator species captured on dandelion and clover flowers in 2012, categorized by tribe or family.

Pollinator species	Dandelion		Clover			
	Spring		Spring		Late summer	
	N	%	N	%	N	%
<b>Apini</b>						
<i>Apis mellifera</i>	18	9.1	104	48.8	110	49.1
<b>Bombini</b>						
<i>Bombus griseocollis</i>	0	0	10	4.7	0	0
<i>Bombus impatiens</i>	0	0	12	5.6	67	29.9
<b>Ceratini</b>						
<i>Ceratina strenua</i>	1	0.51	0	0	0	0
<b>Nomadini</b>						
<i>Nomada articulata</i>	0	0	1	0.47	0	0
<i>N. imbricata</i>	2	1	1	0.47	0	0
<b>Andrenidae</b>						
<i>Andrena barbara</i>	0	0	2	0.94	0	0
<i>A. commoda</i>	1	0.51	2	0.94	0	0
<i>A. cressonii</i>	0	0	1	0.47	0	0
<i>A. imitatrix</i>	1	0.51	0	0	0	0
<i>A. morrisonella</i>	1	0.51	1	0.47	0	0
<i>A. nasonii</i>	3	1.5	0	0	0	0
<i>A. perplexa</i>	1	0.51	3	1.4	0	0
<i>A. pruni</i>	0	0	1	0.47	0	0
<i>A. wilkella</i>	0	0	3	1.4	0	0
<i>Caliopsis andreniformis</i>	0	0	0	0	2	0.9
<b>Halictidae</b>						
<i>Anthidium o. oblongatum</i>	0	0	0	0	5	2.2
<i>Halictus c. confusus</i>	0	0	1	0.47	4	1.8
<i>H. ligatus</i>	3	1.5	1	0.47	0	0
<i>H. rubicundus</i>	0	0	0	0	1	0.4
<i>Lasioglossum sp.</i>	3	1.5	0	0	0	0
<b>Megachilidae</b>						
<i>Megachile rotunda</i>	0	0.	0	0	1	0.4
<i>Osmia cordata</i>	0	0	1	0.47	0	0
<b>Lycaenidae</b>						
<i>Cupido commyntas</i>	1	0.51	2	0.94	5	2.2
<b>Hesperiidae</b>						
<i>Hesperia comma</i>	28	14.2	9	4.2	14	6.3
<b>Nymphalidae</b>						
<i>Junonia coenia</i>	0	0	0	0	4	1.8
<i>Speyeria sp.</i>	0	0	0	0	8	3.6
<b>Pieridae</b>						
<i>Colias sp.</i>	1	0.51	0	0	0	0
<b>Syrphidae</b>	133	67.5	58	27.2	3	1.3
<b>TOTAL</b>	197		213		224	

There were no significant differences between diversity on clover versus dandelion (Wilcoxon ranked test,  $P=0.31$ ) or on clover in the spring versus late summer ( $P=0.06$ ). The dominant species collected from dandelion flowers in spring 2012 were flower flies, the silverspotted skipper *Hesperia comma* L. (Hesperiidae), and honey bees which together accounted for 90.8% of the pollinator assemblage (Table 6.2). Honey bees constituted nearly half of the specimens from flowering white clover during the spring (7–9 May) sample period and were collected at all lawn sites (Table 6.2, Figure 6.2), as were flower flies, *B. impatiens*, and the brown-belted bumble bee *Bombus griseocollis* DeGeer. Various species of solitary bees, butterflies, and skippers, *H. comma*, also visited spring-blooming clover in the sampled lawns (Table 6.2). Social bees, more specifically *A. mellifera* and *B. impatiens*, dominated the pollinator assemblage of white clover in late summer, with those species accounting for 79% of the total collections (Table 6.2).

There were proportionately more of the social bees, *A. mellifera*, *B. griseocollis* and *B. impatiens*, on clover flowers than on dandelion flowers (Table 6.2;  $F_{1,7}=16.5, 7.1, 3781$ ;  $P<0.01, 0.05, 0.001$ , respectively). Flower flies were relatively more abundant on dandelions than on white clover (Table 6.2,  $F_{1,7}=19.5, P<0.01$ ). Flowering clover was visited by relatively more *B. griseocollis* and *B. impatiens* ( $F_{1,7}=7.1, 9.5$ , respectively,  $P<0.05$ ) and fewer syrphid flies ( $F_{1,7}=39.4, P<0.001$ ) in late summer than in the spring.

### **Pollinator Assemblages of White Clover in Lawns across an Urban-Rural Gradient**

In total 986 insect pollinators representing 26 different species were collected and identified from white clover blooms across the 18 lawn sites (Table 6.3). Species richness

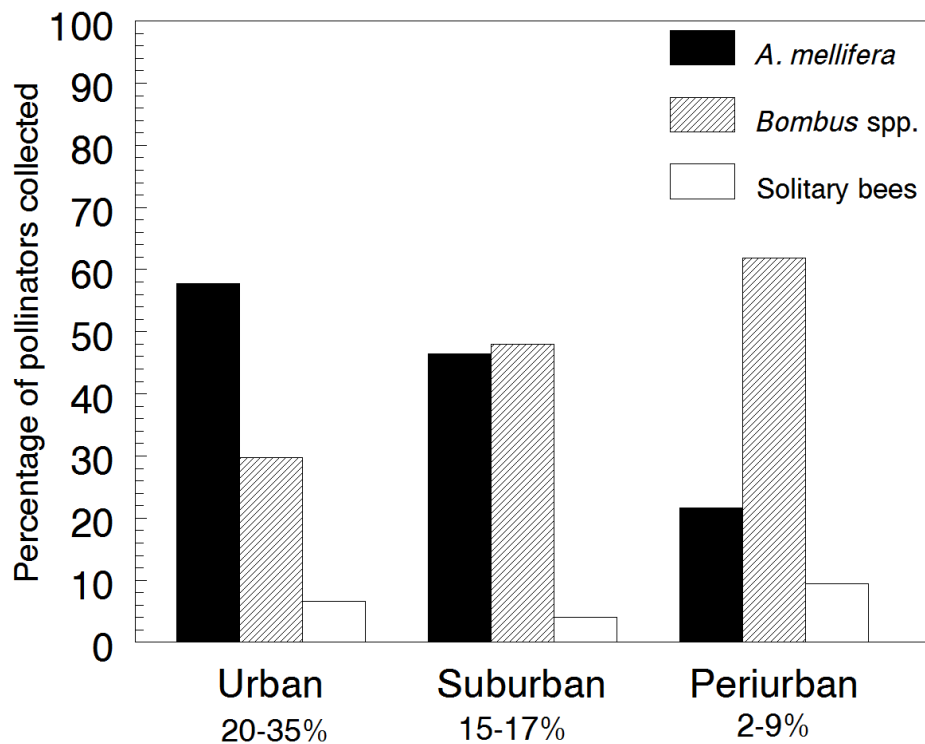


Figure 6.2. Relative abundance of honey bees (*A. mellifera*), bumble bees (*Bombus* spp.), and solitary bees (Andrenidae, Halictidae, and Megachilidae) in 50-pollinator samples collected from flowering white clover in 18 lawn sites classified as urban, suburban, or periurban-rural. Percentages under X-axis correspond to amount of impervious surface within a 200 m radius of the sampled lawn.



Table 6.3. Total pollinators collected from flowering white clover at 18 lawn sites in urban, suburban, or periurban settings based on amount of impervious surface area (20–35, 15–17, or 1–9 %, respectively) within a 200-m radius.

<b>Classification along Urban-Rural Gradient</b>			
Pollinator type	Urban (7 Sites)	Suburban (4 Sites)	Periurban (7 Sites)
<b>Apini</b>			
<i>A. mellifera</i>	236	94	82
<b>Bombini</b>			
<i>Bombus impatiens</i>	76	60	136
<i>B. griseocollis</i>	30	21	69
<i>B. bimaculatus</i>	15	14	29
<i>B. fervidus</i>	0	1	0
<i>B. perplexus</i>	0	1	0
<b>Xylocopini</b>			
<i>Xylocopa virginica</i>	0	0	1
<b>Ceratini</b>			
<i>Ceratini sp.</i>	1	0	0
<b>Andrenidae</b>			
<i>Andrena wilmattae</i>	0	0	1
<i>A. perplexa</i>	1	0	0
<i>A. wilkella</i>	11	3	17
<b>Halictidae</b>			
<i>Agapostemnon</i>	1	0	0
<i>Viscerans</i>			
<i>Halictus rubicundis</i>	3	2	10
<i>H. ligatus</i>	0	0	3
<i>H. confuses</i>	1	0	1
<i>Lasioglossum sp.</i>	1	0	1
<b>Megachilidae</b>			
<i>Anthidium oblongatum</i>	4	2	2
<i>Megachile rotunda</i>	2	1	0
<i>M. mendica</i>	2	0	0
<i>M. exilis</i>	1	0	0
<i>Osmia cordata</i>	0	0	1
<b>Vespidae</b>			
<i>Polistes dominula</i>	14	1	5
<b>Lepidoptera</b>			
<i>Cupido comyntas</i>	3	1	5
<i>Pieris rapae</i>	5	0	6
<i>Colias philodice</i>	0	1	0
<i>Nymphalidae</i>	1	0	9
<b>Total Pollinators</b>	407	202	377

ranged from 2–12 per site. Five species, *A. mellifera*, *B. impatiens*, *B. griseocollis*, the two-spotted bumble bee *Bombus bimaculatus* Cresson, and *A. wilkella*, were collected from at least 13 of the 18 sites. Other bee, wasp, and butterfly species were more sporadically collected (Table 6.3).

Species richness of pollinators visiting flowering white clover in urban, suburban, and periurban-rural lawns was similar regardless of the amount of impervious surfaces within a 200 or 500 m radius (Kruskal-Wallis test;  $H = 0.14, 0.13$ , respectively;  $df = 2, P > 0.9$ ). Pollinator species diversity (Simpson's 1-D) also did not differ between weedy lawns in those types of settings ( $H = 3.03, P = 0.22$ ), nor was it related to differences in percentage of surrounding impervious surface area across the 18 sites ( $r^2 = 0.003$ , ANOVA for slope of regression:  $F_{1,9} < 0.1, P > 0.8$ ).

There were, however, differences in the makeup of the bee assemblages visiting flowering white clover between urban, suburban, and periurban-rural lawns (Figures 6.2, 6.3). Solitary bees comprised a similarly low percentage of the overall bees collected from flowering clover regardless of lawn setting. Honey bees dominated in the samples collected from urban lawns, whereas bumble bees were proportionately more abundant on lawns in periurban-rural settings (Kruskal-Wallis  $H = 8.3, 7.8$  for *A. mellifera* and *Bombus* spp., respectively;  $P < 0.05$ ; Figure 6.2). The same pattern, i.e., proportionately more honey bees and fewer bumble bees with increasing impervious surface area around the lawn sites, was evident across the urbanization gradient (Figure 6.3).

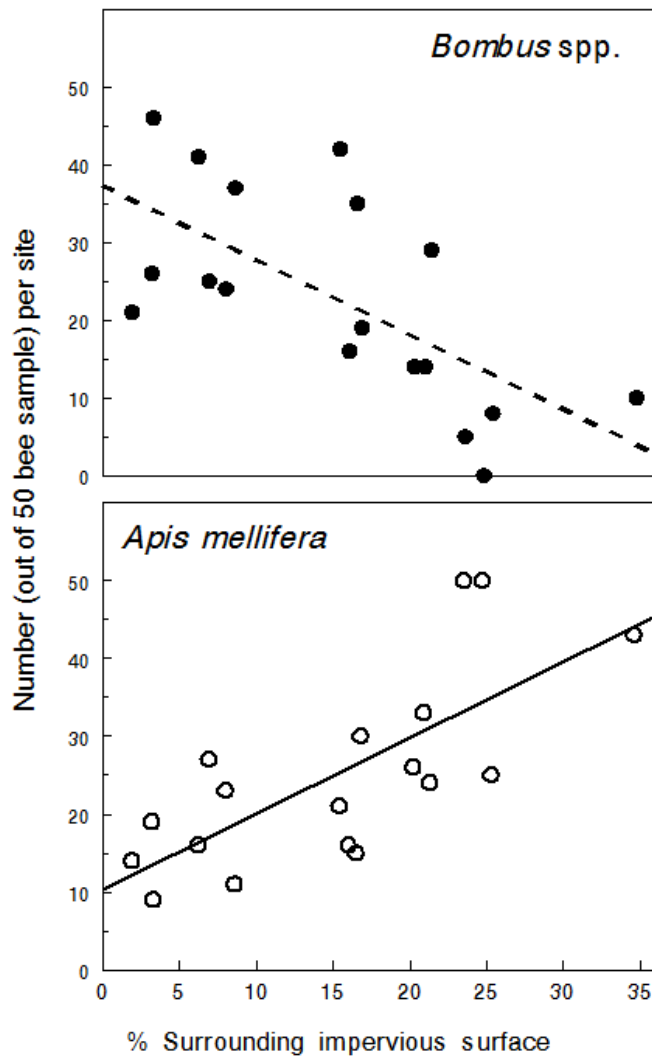


Figure 6.3. Number of *A. mellifera* or *Bombus* spp. in 50-pollinator samples from flowering white clover in 18 lawns surrounded by differing amounts of impervious surface within a 200 m radius. Linear regression;  $r^2 = 0.43, 0.53$ ; ANOVA for slope of regression:  $F = 12.1, 18.4$ ;  $P < 0.005, < 0.001$ , for honey bees and bumble bees, respectively. Regression equations: *Bombus* =  $38.14 - 0.86 \cdot \text{pct}$ ; *Apis mellifera* =  $13.32 + 0.63 \cdot \text{pct}$ .

## **Discussion**

In the United States there are strong normative pressures to maintain lawns as lush, dark green, low-cut swards of a single grass species (Robbins and Sharp 2003, Held and Potter 2012, Blaine et al. 2012). Many homeowners view plants such as dandelion and clover as weeds to be suppressed with herbicides or physically removed. Nevertheless, interest in more sustainable lawn care practices is growing (Leslie and Knoop 1989, Carpenter and Meyer 1999, Sandberg and Foster 2007, Dobbs and Potter 2014), along with public awareness and concern about declining populations of charismatic insects such as honey bees and monarch butterflies, *Danaus plexippus* L. (Risnik 2013, Walsh 2013).

Our study highlights the potential value of low input lawns with some flowering weeds as a resource for urban pollinators. Indeed, we documented > 50 different species of insect pollinators visiting white clover and dandelion, with similar diversity on lawns in rural and urbanized areas. Awareness of that biodiversity could encourage some homeowners to be more tolerant of flowering clover intermixed with conventional turf grasses. Clover may be better suited than dandelion for bee conservation lawns because it blooms throughout the growing season, is more uniform, and tolerates low mowing. Our results indicate that compared to dandelions, white clover attracts similar numbers of solitary bees, and is relatively more attractive to social bees. Mixed clover lawns are already being promoted for their ability to withstand drought, thrive in poor soil, and supply nitrogen to other plants (Sincik and Acikgoz 2007, McCurdy et al. 2013). Promoting their added value as a resource for pollinators could add impetus to cultivation of clover as a lawn plant, and greater acceptance of its unintended presence in lawn

settings. Such lawns potentially could also serve as corridors for movement of urban pollinators between remaining patches of natural habitat and gardens or other plantings dedicated to pollinator conservation.

Increased public awareness that pollinators forage on lawn weeds may also help to encourage more selective use of lawn insecticides (Larson and Potter 2013). For example, the neonicotinoid insecticides used to control root-feeding grubs and other pests (Held and Potter 2012) are harmful to bumble bee colonies if workers forage on flowering lawn weeds that have been inadvertently sprayed (Gels et al. 2002, Larson et al. 2013). Some weeds, including white clover and violets, *Viola sororia* Wild, are difficult to eliminate with herbicides (Ricigliano 2013) so some patches may remain even on high-input lawns. By considering what species visit those weeds, and when during the growing season, timing of lawn care applications might be adjusted to better protect pollinators. Simple cultural practices such as mowing flower heads before or after pesticide applications, using more target-selective insecticides (Larson et al. 2013) and educational material aimed at stakeholders (e.g. turf care providers, extension agents, and homeowners Larson and Potter 2013) could also promote urban bee conservation.

Further work is needed to understand how the structure and management of urban landscapes affect pollinator communities. For example, the extent of impervious surfaces surrounding floral resources may adversely affect some species but not others (e.g., Bates et al. 2011). Indeed, our finding proportionately more honey bees, and fewer bumble bees, on white clover in urban versus more rural settings is consistent with Ahn   et al. (2009) who found decreasing diversity of bumble bees in gardens along a gradient of increasing urbanization. There may be ways to integrate lawns, gardens, woody

landscape plants, and fragments of natural habitat to better support urban pollinator communities.

Finally, low-input lawns may offer opportunities for an increasingly urbanized public to observe and learn about pollinators. Rather than fearing lawn-foraging bees as a sting hazard to be eliminated, perhaps people can learn to appreciate them, or even encourage their presence. Although the prospect of converting the traditional American lawn to a mixed-plant meadow is unlikely to be embraced by most suburban residents, awareness of the diverse pollinator assemblages of flowering lawn weeds might help nurture a sociocultural shift toward more pollinator-friendly and sustainable lawn care practices.

## **CHAPTER SEVEN**

### **Summary and Implications**

Turf managers are seeking ways to implement more integrated pest management strategies, including conservation biological control. Pest-selective insecticides, such as the anthranilic diamide chlorantraniliprole, are conducive to these industry wide initiatives. This project was the first to assess the field impacts of the relatively new chlorantraniliprole on non-target invertebrates in turf. I sought to contribute information about the impacts of representatives of each of the major classes of preventive insecticides on beneficial organisms including natural enemies, decomposers, and pollinators. In addition, my research contributed to extension teaching by giving managers access to concrete, research supported recommendations they could adopt. This chapter summarizes the key findings of my doctoral research.

### **Chapter Two Summary**

Chlorantraniliprole, the first anthranilic diamide insecticide labeled for turf, combines strong selective activity against key pests with low vertebrate toxicity. The hypothesis that it is less disruptive to beneficial invertebrates and their ecosystem services than are other prevailing insecticide classes was tested. Plots in golf course settings were treated with chlorantraniliprole, or with a representative neonicotinoid (clothianidin), pyethroid (bifenthrin) or a premix combination (clothianidin–bifenthrin) formulation. Non-target effects were assessed via pitfall traps (epigeal predators), Tullgren funnel extraction (soil microarthropods), hand sorting (earthworms), counting ant mounds and earthworm casts on tees and putting greens, assessing predation on sentinel pest eggs and comparing grass clipping decomposition in treated versus

untreated turf. Chlorantraniliprole had little or, in most cases, no impact on predatory or soil invertebrates, predation or decomposition. Each of the other insecticides temporarily reduced abundance and activity of one or more predator groups. Clothianidin and the clothianidin–bifenthrin combination retarded grass clipping decomposition, and the combination suppressed earthworms and casts more than did carbaryl, a toxic standard. Chlorantraniliprole is compatible with conservation biocontrol and a good fit for industry initiatives to use relatively less toxic pesticides. One qualification is that its use on golf courses may require targeted management of ant mounds and earthworm casts that are suppressed as a side effect of less selective insecticides.

### **Chapter Three Summary**

Many turf managers prefer to control foliage- and root-feeding pests with the same application, so-called multiple-targeting, using a single broad-spectrum insecticide or a premix product containing two or more active ingredients. We compared the impact of a clothianidin, a premix (clothianidin + bifenthrin), and chlorantraniliprole, the main insecticide classes used for multiple targeting, on four species of beneficial insects:

*Harpalus pennsylvanicus*, an omnivorous ground beetle, *Tiphia vernalis*, an ectoparasitoid of scarab grubs, *Copidosoma bakeri*, a polyembryonic endoparasitoid of black cutworms, and *Bombus impatiens*, a native bumble bee. Ground beetles that ingested food treated with clothianidin or the premix suffered high mortality, as did *C. bakeri* wasps exposed to dry residues of those insecticides. Exposure to those insecticides on potted turf cores reduced parasitism by *T. vernalis*. Bumble bee colonies confined to forage on white clover (*Trifolium repens* L.) in weedy turf that had been treated with clothianidin or the premix had reduced numbers of workers, honey pots, and immature



bees. Premix residues incapacitated *H. pennsylvanicus* and *C. bakeri* slightly faster than clothianidin alone, but otherwise we detected no synergistic or additive effects.

Chlorantraniliprole had no apparent adverse effects on any of the beneficial species.

#### **Chapter Four Summary**

Maintaining bee-friendly habitats in cities and suburbs can help conserve the vital pollination services of declining bee populations. Despite label precautions not to apply them to blooming plants, neonicotinoids and other residual systemic insecticides may be applied for preventive control of lawn insect pests when spring-flowering weeds are present. Dietary exposure to neonicotinoids adversely affects bees, but the extent of hazard from field usage is controversial. Colonies of *Bombus impatiens* were exposed to turf with blooming white clover that had been treated with clothianidin, a neonicotinoid, or with chlorantraniliprole. The sprays were applied at label rate and lightly irrigated. After residues had dried, colonies were confined to forage for six days, and then moved to a non-treated rural site to openly forage and develop. Colonies exposed to clothianidin-treated weedy turf had delayed weight gain and produced no new queens whereas those exposed to chlorantraniliprole-treated plots developed normally compared with controls. Neither bumble bees nor honey bees avoided foraging on treated white clover in open plots. Nectar from clover blooms directly contaminated by spray residues contained  $171 \pm 44$  ppb clothianidin. Notably, neither insecticide adversely impacted bee colonies confined on the treated turf after it had been mown to remove clover blooms present at the time of treatment, and new blooms had formed. Our results validated EPA label precautionary statements not to apply neonicotinoids to blooming nectar-producing

plants if bees may visit the treatment area. Chlorantraniliprole usage on lawns appears non-hazardous to bumble bees.

## **Chapter Five Summary**

We tested the extent to which systemic neonicotinoid insecticides might be translocated into nectar of flowering weeds or grass guttation droplets, which could pose a hazard to non-target insects including bees. Imidacloprid and clothianidin were applied to lawn turf with white clover, followed by irrigation. Residues in clover blooms that were directly contaminated by the sprays, or that formed after mowing, were analyzed by LC-MS/MS. Similar methods assessed imidacloprid residues in creeping bentgrass guttation. Bioactivity was determined by feeding the exudates to *Orius insidiosus*, a small predatory bug. Nectar from sprayed clover blooms contained 5493–6588 ng/g imidacloprid or 2882–2992 ng/g clothianidin and was toxic to *Orius*. Residues in new blooms formed a few days after the first mowing were > 99.5% lower and nontoxic to the bugs. Guttation from field-grown bentgrass contained 88 or 23 ng/g imidacloprid at 1 and 3 weeks after treatment, respectively, and was nontoxic to *Orius*. Systemic levels of neonicotinoids in white clover nectar and creeping bentgrass guttation are relatively low and transitory. Hazard to non-target insects via nectar of flowering weeds in treated lawns can be mitigated by adherence to label precautions and mowing to remove blooms if flowering weeds are inadvertently sprayed.

## **Chapter Six Summary**

Flowering weeds, though often deemed undesirable in turfgrass lawns, may provide food resources for declining pollinator populations in urbanized landscapes. The

pollinator assemblages visiting flowering dandelion (*Taraxacum officinale* Wigg) and white clover (*Trifolium repens* L.) in lawn settings of similar character in central Kentucky USA were compared to document their biodiversity and identify species that could be exposed when such sites are treated with pesticides. Species richness and diversity of pollinators visiting white clover at 18 lawn sites along a gradient of increasing urbanization was compared to test how the proportion of impervious surface to green space in surrounding areas affects these pollinator communities. More than 50 pollinator species, including hover flies (Syrphidae), honey bees (*Apis mellifera*), six species of bumble bees (*Bombus* spp.) and 26 species of native solitary bees (Andrenidae, Halictidae, Megachilidae) were hand-netted from the aforementioned weed species. Hover flies, honey bees, and solitary bees predominated on dandelions whereas proportionately fewer syrphids and more social bees (*A. mellifera* and *Bombus* spp.) visited white clover, especially in summer. Lawns with clover supported similar species richness and diversity of pollinators across the urban-rural gradient, although honey bees were proportionately more abundant, and representation of bumble bees decreased, with increasing urbanization. Flowering weeds in lawns help to support relatively diverse pollinator assemblages that can provide pollination services to native and cultivated plants in urbanized areas, as well as educational opportunities for fostering a more environmental lawn care ethic.

## **Implications**

The Green Industry will be increasingly affected by new regulatory measures and the public's concerns about the safety of pesticides, including those used on lawns, golf courses, and sports fields. Many turf managers are motivated by these pressures and by

their own beliefs, to use more environmentally conscious turf care methods. At the same time, it is important to recognize that the livelihoods of professional turf managers are dependent upon delivering the quality of turfgrass their clientele demand. Turf care providers will favor those insecticides they know will work. In my experience, however, they are also receptive to new insecticidal chemistries or modified treatment protocols that mitigate environmental impacts and preserve beneficial processes such as conservation biological control.

This work, highlighting that chlorantraniliprole does not affect non-target beneficial organisms, may help turf managers to justify its higher cost, relative to older less selective insecticides, to customers, supervisors, or greens committees. As long as preventive treatments dominate the market, it is in the best interests of managers to choose products that work but do not disrupt beneficial organisms. It was also important to document the potential drawbacks, such as not suppressing mounding or casting by ants and earthworms, on golf greens and tees, when switching to more pest-selective chemistries. Hopefully these results will allow superintendents to better know what to expect before they adopt the use of chlorantraniliprole, or related products, on their golf courses.

One of my goals during this research was to incorporate field trials and to provide more realistic information about neonicotinoid effects on pollinating insects. These insecticides are toxic to bees but this work highlights that, in turf at least, there are ways neonicotinoids can be used without hazard to pollinators. My results have been of interest to many green industry representatives. Part of my graduate experience was sharing my work with these managers at extension events where I was able to teach about pollinator

conservation and hopefully convince growers to follow label instructions and be conscious of how their actions can impact bees. It will be important as we head into a period of tighter pesticide regulation that the turf industry can defend its practices. Bee conservation will be a driving issue for the lawn and landscape industries in the next decade or more. I believe my work will help managers to make better informed decisions.

Another contribution of this research was to highlight that common flowering lawn weeds provide resources to diverse types of bees and other pollinators. Hopefully, that may encourage more tolerance of these plants or even support a change in attitude wherein they are viewed positively someday. As concern grows about the welfare of bees and other pollinators, cities and private citizens are implementing bee gardens to provide food and shelter for urban bees. Clover and other blooming lawns weeds could become an important component of urban pollinator conservation by serving as food in the corridors between these planted gardens. In addition, by implementing more environmental conservation areas and using our own yards, more could be done to teach an increasingly urban populace to about insects and pollination. My findings about pollinator diversity on weeds won't bring immediate wholesale shifts in attitudes about weed management, but it could be part of the first steps in a different direction.

I am hopeful that the research presented in this dissertation will clarify the effects of systemic insecticides used in turf so managers can make more informed decisions in the future. I believe that some of the methods we used to assess colony-level impacts could become important parts of the regulatory process in the future. Field based evaluations, with realistic treatments of insecticides and exposure for non-target insects should be considered by the US EPA and the companies that produce insecticides.

Preemptively finding out about possible negative effects and mitigation strategies will assist the green industry as it transitions towards a more sustainable future.

### **Future Directions**

Future research on the ecotoxicology of the insecticides assessed here as well as others will need to be implemented in field and should also go beyond assessments of short-term acute effects on populations. Long-term reproductive effects on earthworms and predators found in turf settings have not been assessed thoroughly. In addition, more work needs to be performed to understand the physiological basis for chlorantraniliprole's selectivity within and between orders of insects.

In the future, pollinator responses to insecticide exposure should include field-based assessments with realistic dosages and ingestion and focus on long term reproductive effects. After measuring concentrations of insecticides in flowering weeds for this dissertation, I believe that it is paramount to determine the movement of neonicotinoid insecticides in woody ornamental plants such as azalea or in treated trees like ash. Understanding the movement and longevity of this class in flowering plants such as these is essential in formulating strategies to protect pollinators from residues. In addition, surveys should be conducted on these plants to determine what pollinator species are at risk. Finally, more needs to be known about the foraging habits and food sources for native, solitary bees.

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## **VITA**

### **Education**

Purdue University, West Lafayette, IN 2005-2009  
Bachelor of Science: Entomology, May 2009

### **Professional experience**

University of Kentucky, Lexington, KY 2009-present  
Graduate Research Fellow  
Purdue University, West Lafayette, IN  
Undergraduate research assistant, Landscape Entomology Lab 2005-2009  
Undergraduate research assistant, CAPS Regulatory Entomology Program 2007

### **Publications**

#### ***Journal Articles (refereed)***

- Larson, J. L.**, A.J. Kesheimer, and D. A. Potter. Pollinator diversity on dandelions and white clover in urban and suburban Lawns. *Journal of Insect Conservation*. In review.
- Larson, J. L.**, C. T. Redmond, and D. A. Potter. 2014. Comparative impacts of an anthranilic diamide, neonicotinoid, and neonicotinoid-pyrethroid premix on beneficial indicator species in turf. *Ecotoxicology*. 23:252–259.
- Larson J.L.**, C.T. Redmond, and D.A. Potter. 2013. Assessing insecticide hazard to bumble bees foraging on flowering weeds in treated lawns. *PLOS ONE* 8(6): e66375. doi:10.1371/journal.pone.0066375
- Larson, J. L.**, C. T. Redmond, and D. A. Potter. 2012. Comparative impact of an anthranilic diamide and other insecticidal chemistries on beneficial invertebrates and ecosystem services in turfgrass. *Pest Management Science* 68: 740–748.
- Blubaugh C.K., V.A. Caceres, I. Kaplan, **J. L. Larson**, C.S. Sadof, and D.S. Richmond. 2011. Ground beetle (Coleoptera: Carabidae) phenology, diversity,

and response to weed cover in a turfgrass ecosystem. *Environmental Entomology* 40: 1093-1101.

### ***Book Chapters***

**Larson, J. L.**, E. K. Dobbs, and D. A. Potter. 2013. "Lilac Borer and Banded Ash Clearwing Borer", in Entomological Society of America's *Wood-Boring Insects Handbook*, JC Chong (ed.). In press.

Dobbs, E. K., **J. L. Larson**, and D. A. Potter. 2013. "Dogwood Borer", in Entomological Society of America's *Wood-Boring Insects Handbook*, JC Chong (ed.). In press.

### **Technical Publications (non-refereed)**

**Larson J.L.** and D. A. Potter. 2013. "The Plight of the Bees", in *Golf Course Management* November 2013 edition, p. 85-88;  
<http://gcmdigital.gcsaa.org/i/199080/88>.

**Larson J.L.** and D.A. Potter. 2013. "Bee Friendly Lawn Care", in *Landscape Management*. December 2013 edition, p. 27-29;  
<http://editiondigital.net/publication/?i=186500>.

### **Scientific Presentations**

#### ***Invited Symposia***

2013: The 61<sup>st</sup> Annual Meeting of the Entomological Society of America. "Pollinating Insects that Visit Lawns and Methods of Conservation." Austin, TX.

2012: The 60<sup>th</sup> Annual Meeting of the Entomological Society of America. "Comparative Ecotoxicology of Turfgrass Insecticides: Impacts on Pollinators, Natural Enemies, and Ecosystem Services." Knoxville, TN.

2010: 58<sup>th</sup> Annual Meeting of the Entomological Society of America. "Ecotoxicology of Turfgrass Insecticides: Impacts on Beneficial Invertebrates." San Diego, CA.

#### ***Submitted presentations***

2011: 59<sup>th</sup> Annual Meeting of the Entomological Society of America. “Lawn Insecticide Impact on Bumble Bee Colonies Assessed Through Realistic Exposure.” Reno, NV.

University of Kentucky Graduate Student Interdisciplinary Conference.

“Comparative Toxicity a Novel Anthranilic Diamide and Other Insecticides on Beneficial and Non-Target Invertebrates in Turf.” Lexington, KY.

2010: 65<sup>th</sup> Annual Meeting of the North Central Branch of the Entomological Society of America “Ecotoxicology of Turfgrass Insecticides: Impacts on Beneficial Invertebrates.” Louisville, KY.

2009: 57<sup>th</sup> Annual Meeting of the Entomological Society of America “Comparative Toxicity of Chlorantraniliprole (Acelepryn), a Novel Anthranilic Diamide, and Other Insecticides on Beneficial and Non-Target Invertebrates in Turf.” Indianapolis, IN.

#### ***Invited Extension Presentations***

2014: Tri-State Green Industry Conference. “Turf IPM: How to Knock Out the Bad Bugs but Keep the Good Bugs.” Sharonville, OH. February.

Purdue Professional Landscape Management School. “Landscape Neonicotinoid Insecticides and Pollinating Insects.” Evansville, IN. January.

2013: Efficient Pest Management Practices Workshop. “Environmental Conservation through Insecticide Stewardship.” Elizabethtown, KY. December.

Ohio Valley Buckeye Horticulture Extension Group Annual meeting. “Urban Bees and Tainted Nectar: How to Keep Pollinators Safe.” Midway, KY. October.

University of Kentucky Turfgrass Research Field Day. “Urban Pollinators and How you can protect them.” Lexington, KY. June

Central Kentucky Ornamentals and Turf Association Annual meeting. “Why Pollinators are in Danger and How Landscape Managers Can Preserve Them.” Lexington, KY. February.



2012: Integrated Pest Management and Best Practices Workshop. “Comparing Turf Insecticides: Impacts on Beneficials and Ecosystem Services.” Georgetown, KY. December.

Kentucky Turfgrass Council- Annual conference. “Turf Insecticides and Pollinating Insects.” Florence, IN. October.

Ohio Valley Buckeye Horticulture Extension Group- Annual meeting. “Pest control & Pollinator Conservation.” Lexington, KY. October.

University of Kentucky Turfgrass Research Field Day. “Lawn Insecticide Use and Pollinators.” Lexington, KY. June.

Kentucky Turfgrass Council- Turf and Landscape Management Short Course. “Pollinating Insects and Turfgrass.” Louisville, KY. February.

Kentucky Turfgrass Council- Turf and Landscape Management Short Course. “Turf Insects of Kentucky: Good Bugs vs. Bad Bugs.” Louisville, KY. February.

Central Kentucky Ornamentals and Turf Association- Annual meeting. “Pollinators and Turfgrass Insecticides” Lexington, KY. February.

2011: Kentucky Turfgrass Council- Annual conference. “Turf Insecticides and Pollinating Insects.” Florence, IN. October.

Ohio Valley Buckeye Horticulture Extension Group- Annual meeting. “Pollinator Conservation.” Lexington, KY. October.

University of Kentucky Turfgrass Research Field Day. “Lawn Insecticides and Pollinators.” Lexington, KY. July.

Bluegrass Golf Course Superintendents Meeting. “Impact of Turf Insecticides on Beneficial Insects” Arlington, KY. June.

Kentucky Turfgrass Council- Turf and Landscape Management Short Course. “Killing Bad Bugs and Saving Good Bugs” Louisville, KY. February.

2010: Kentucky Turfgrass Council- Annual conference. “Turf Insecticides and Beneficial Insects.” Florence, IN. October.

Ohio Valley Buckeye Horticulture Extension Group- Annual meeting. “Beneficial Insects in Turf and How to Conserve Them” Lexington, KY. October.

University of Kentucky Turfgrass Research Field Day. “The Heroes of Turfgrass: Beneficial Insects and How Insecticides Impact Them.” Lexington, KY. July.  
Central Kentucky Ornamentals and Turf Association- Annual meeting. “Making a Greener Green Industry” Lexington, KY. February.  
2009: Ohio Valley Buckeye Horticulture Extension Group- Annual meeting.  
“Chlorantraniliprole and Non-Target Insects” Lexington, KY. October.

## **Grants**

2013: University of Kentucky Nursery/Landscape Endowment Grant: *Pre-arming the Landscape and Nursery Industries for the Impending Regulatory Storm over Systemic Insecticide Hazard to Bees*. **Jonathan L. Larson**, \$2,500

## **Leadership roles**

Ohio Valley Entomological Association

*President*- 2011-2012

H. Garmin Graduate Student Entomology Club- University of Kentucky

*President*- 2010-2011

Thomas Say Entomological Society- Purdue University

*President*- 2007-2009

## **Service**

Student-Faculty Liaison- University of Kentucky Entomology Department

August 2011- August 2013

Committee Service

Student Seminar Evaluation Committee- January 2013-May 2013

Symposium Organizer/Moderator

Turf and Ornamentals Symposium, Annual Meeting of the Entomological Society of America, Austin, TX. 2013.

Symposium Moderator

Turf and Ornamentals Symposium, Annual Meeting of the Entomological Society of America, San Diego, CA. 2010.

### **Awards**

2013: Outstanding PhD Student Award, University of Kentucky College of Agriculture, Food and Environment

2011: First place Master's Division Oral Presentation. Ohio Valley Entomological Association.

First place Poster Presentation. University of Kentucky Graduate Student Interdisciplinary Conference

2010: First place Master's Division Oral Presentation. Ohio Valley Entomological Association.

### **Professional Associations**

Ohio Valley Entomological Association

Entomological Society of America

Gamma Sigma Delta